

ZOO AND WILD ANIMAL MEDICINE

Current Therapy

6



MURRAY E. FOWLER
R. ERIC MILLER



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Preface

With the sixth volume of **Zoo and Wild Animal Medicine** we return to the *Current Veterinary Therapy* format. Topics were selected to address current issues. Authors were selected for their experience and expertise in captive wild animal medicine or in the field with free-ranging wildlife.

The sixth volume reflects a world view of some of the special challenges that face wild-animal veterinarians as they seek to assist in the preservation and conservation of wild animals. Countries represented include Australia, Austria, Canada, China, England, Germany, Mexico, The Netherlands, Northern Ireland, Scotland, United Arab Emirates, and the United States.

Some of the topics address current problems, such as chronic wasting disease in cervids and tuberculosis in free-ranging deer and elephants. Other topics describe newly emerging or newly recognized diseases, such as paramyxovirus in bats and protozoal encephalitis in marine mammals.

One topic that is addressed and needs to be considered by all medical professionals is the growing awareness that wildlife, domestic animals, and humans

are all subject to pressures from the same or similar infectious agents and environmental stresses. All animals interrelate with one another. Determining how disease agents circulate in the world requires a holistic approach to medicine.

The medical management of species or groups of animals that may not have been thoroughly discussed elsewhere includes bustards and chamois. A topic of vital concern to the world is the potential for a pandemic of avian influenza to wreak havoc on humans and domestic and wild animal populations. The avian chapter has been continually updated throughout the production of this book to keep current on what is happening worldwide with avian influenza.

This volume discusses animal medical management of selected species in all the major vertebrate groups. It is hoped that there will be chapters of interest to all readers who are concerned about the preservation and conservation of the world's fauna.

Murray E. Fowler
R. Eric Miller

Acknowledgments and Dedication

Our thanks to the more than 50 authors who contributed 57 topics to the sixth volume of *Zoo and Wild Animal Medicine*. This is especially significant because all of the royalties support research for wild animals, with none going to the authors or editors. Wild animals deserve our support. Thanks are also due to the many researchers who are gathering data on the biology and medicine of wild animals.

Acknowledgment and thanks are expressed to the institutions that supported the authors as they completed their writing tasks.

Once again, we thank our wives, Audrey and Mary Jean, for vocal and moral support while we took time away from family activities to complete the task of editing and bringing it all together.

This volume is dedicated to all veterinarians who use their time, talents, expertise, and finances to care for and study wild animals throughout the world.



Color Plate 1 A killer whale (*Orcinus orca*) breaching. A continual challenge for wildlife veterinarians and biologists is to provide an optimal environment in captivity and also to work for conservation in the wild. (This image does not appear in the text.)



Color Plate 2 Cheetahs (*Acinonyx jubatus*). Lack of genetic diversity hampers providing optimal conservation efforts for this species. (This image does not appear in the text.)



Color Plate 3 White tiger (*Panthera tigris*). This color variation is accompanied by decreased immune competence. (This image does not appear in the text.)



Color Plate 4 A blue and yellow macaw (*Ara ararauna*). Macaws are popular pet birds, but serious concerns exist regarding conservation issues with pet sales. (This image does not appear in the text.)

CHAPTER 1

West Nile Virus in Birds and Mammals

DOMINIC TRAVIS

ETIOLOGY

West Nile virus (WNV) is an arthropod-borne virus (arbovirus) in the family *Flaviviridae*, genus *Flavivirus*—Japanese encephalitis antigenic complex—that includes Alfuy, Cacipacore, Japanese encephalitis, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Rocio, Stratford, Usutu, West Nile, and Yaounde viruses. Flaviviruses share a common size (40–60 nm), symmetry (enveloped, icosahedral nucleocapsid), nucleic acid (positive-sense, single-stranded RNA of ~10,000–11,000 bases), and appearance on electron microscopy. The close antigenic relationship of the flaviviruses, particularly those belonging to the Japanese encephalitis complex, accounts for the cross-reactions observed in diagnostic serologic assays.³¹

HISTORY AND DISTRIBUTION

WNV has been described in Africa, Europe, the Middle East, West and Central Asia, Oceania (subtype Kunjin), and most recently in the Western Hemisphere. It was first isolated from a febrile adult woman in the West Nile District of Uganda in 1937, and its ecology was first characterized in Egypt in the 1950s. The virus became recognized as a cause of severe human meningitis or encephalitis in Israel in 1957. Equine disease was first noted in Egypt and France in the early 1960s.^{33,40,57} Recent outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996–1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States (U.S.) in 1999–2003,^{7,10,19,58} and Israel in 2000.³³ Epizootics have occurred in horses around the Mediterranean (Morocco in 1996, Italy in 1998, France in 2000),⁵⁷ and in the U.S. in 1999–2001.^{61,78} A thorough review of pre-North American WNV ecologic history was published by Komar.⁴⁰

WNV was first found in North America in New York City in humans, equines, and free-ranging and captive wildlife in 1999.^{7,40,58,71} Since 1999 in the U.S., more than 20,000 humans have been infected, causing more than 700 deaths, and more than 23,000 equine cases and hundreds of thousands of avian cases have been reported. Most cases occur in North America during the summer and fall between July and October, with peaks in August and September. Spread across North America to all 48 contiguous states and seven Canadian provinces has been documented in twice-weekly summary reports available on the U.S. Centers for Disease Control and Prevention (CDC) World Wide Web site* and by interactive maps collated by the U.S. Geologic Survey.[†] In Canada, Health Canada summarizes WNV activity.[‡] Since 1999, surveillance data have shown WNV activity in the Cayman Islands in 2001 (CDC); birds in Jamaica²³ and Guadeloupe⁶⁴ in 2002; horses, humans, and wildlife in Mexico in 2002^{6,24,49}; and birds in the Dominican Republic in 2003⁴¹ and in Puerto Rico and El Salvador in 2004.¹⁷

TRANSMISSION

The arboviral encephalitides are zoonotic, being maintained in complex life cycles involving a nonhuman primary vertebrate host and a primary arthropod vector. Transmission occurs between susceptible vertebrate hosts by blood-feeding arthropod mosquitoes, sand flies, ceratopogonids, “no-see-ums,” and ticks.^{1,2,48,68} Infection usually occurs as a result of a mosquito bite while taking a blood meal. Normal transmission cycles usually remain undetected until humans or other mammals become “accidentally” infected, potentially

*<http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm>.

†<http://westnilemaps.usgs.gov/index.html>.

‡<http://www.hc-sc.gc.ca/english/westnile/>.

as the result of some ecologic change. Humans and domestic animals may develop clinical illness but usually are incidental or “dead-end” hosts because they do not produce significant viremia and thus do not contribute significantly to the transmission cycle.

Since 1999, more than 60 separate species of mosquito have been positive (virus isolated, RNA or antigen detected) through national surveillance.* Although not all these are competent vectors, the predominant species testing positive are *Culex* spp. The discovery that hybrid *Culex* mosquitoes sometimes feed on both humans and birds resulted in a focus on potential “bridge vectors.”²⁷ One risk assessment of mosquito feeding characteristics identified *Culex pipiens* and *C. restuans* as the most competent vectors for humans.³⁷ A 5-year analysis of mosquito data in Connecticut revealed that *Culex* spp. were the most prevalent carriers from July to September, playing a roll in early-season enzootic transmission and late-season epizootic amplification in wild birds. *Culex restuans* was most prevalent in June and July and may play an important role in enzootic transmission and amplification in wild birds early in the season. *Culiseta melanura* was found to be the major orniphilic species and may play a major role in amplification among birds. *Aedes vexans* may play a significant role in transmission to mammals.²

Other, non–arthropod-borne routes of transmission have been reported. New transmission routes in humans include infection through contaminated blood products and transfusion^{13,63} and organ transplantation,¹⁴ maternal transmission through breast milk and intrauterine transmission,¹² and occupational exposure through laboratory “sharps.”¹¹ Experimentally, infection has been demonstrated after oral exposure in cats fed infected mice and birds.^{3,42} Oral exposure to horse meat is the hypothesized route of transmission for infected alligators.⁵⁵ Fecal shedding was identified as a potential route of transmission after experimental direct transmission between cage mates in crows (*Corvus brachyrhynchos*), blue jays (*Cyanocitta cristata*), black-billed magpies (*Pica pica*), and ring-billed gulls (*Larus delawarensis*).^{42,54} Experimental and natural direct transmission also occurred between geese.^{4,75} The importance of these transmission routes is unknown but thought to be of secondary significance compared with arthropod-borne transmission for amplification and spread of the disease.

INTRODUCTION INTO WESTERN HEMISPHERE

The WNV strain first identified in New York was closely related to that recently isolated in Israel.^{33,46} Although the route of introduction is not known, hypotheses include release of infected vectors or hosts through international commerce or travel. Geographic spread via introduction through migratory birds has been hypothesized^{51,65,66} worldwide but is unlikely in the case of introduction into North America. A quantitative risk assessment of pathways by which WNV could reach Hawaii suggests that the most viable hosts for introduction are mosquitoes, rather than birds or other hosts. Viable routes of introduction include transfer of infected hosts via plane or boat, and introduction through migratory birds could not be quantified in this case.³⁶

DIAGNOSIS

Cases are confirmed by combining clinical and laboratory criteria. Standard clinical and laboratory case definitions have been derived for humans^{7,9,30,52,63} and are updated periodically on the CDC website.*

Arboviral infections may be asymptomatic or may result in febrile illnesses of variable severity sometimes associated with central nervous system (CNS) involvement (aseptic meningitis, myelitis, and encephalitis). Arboviral *meningitis* is usually characterized by fever, headache, stiff neck, and pleocytosis in cerebrospinal fluid (CSF). Arboviral *myelitis* is usually characterized by fever and acute paresis or flaccid paralysis. Arboviral *encephalitis* is usually characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction. Nonneuroinvasive syndromes include myocarditis, pancreatitis, or hepatitis. In addition, arboviral infections may cause febrile illnesses (“West Nile fever”) with headache, myalgias, arthralgias, and sometimes accompanied by skin rash or lymphadenopathy.

Laboratory confirmation consists of one of the following criteria:

1. Fourfold or greater increase in virus-specific serum antibody titer.
2. Isolation of virus from, or demonstration of specific viral antigen or genomic sequences in, tissue, blood, CSF, or other body fluid.

*<http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm>, accessed January 2006.

*http://www.cdc.gov/epo/dphsi/casedef/arboviral_current.htm, accessed January 2006.

3. Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA).
4. Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition).

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus may be misleading, as in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both these viruses occur. In areas where WNV has circulated in the recent past, the coexistence of WNV-specific IgM antibody and illness in a given case may be coincidental and unrelated. In those areas the testing of serially collected serum specimens assumes added importance.

Most cases in animals are defined through isolation of the virus or detection of genetic material postmortem. Virus isolation is the “gold standard” but takes time, which limits its use as a rapid surveillance tool. Immunohistochemistry (IHC) and reverse-transcription, nested polymerase chain reaction (RT-PCR) tests are used for detection of antigen, and an antigen-capture assay is also commercially available. No definitive list exists for the most effective combination of tissue and test methodology by species, but some optimal combinations have been reported.^{82,83} In general, virus is best detected in kidneys, brains, and hearts, as well as on oropharyngeal or cloacal swabs antemortem. The success of IHC depends greatly on tissue selection (heart, kidney, liver, lung); brain tissue is best for virus isolation; and RT-PCR is generally the most sensitive test for all tissues, with few reported exceptions.* Recently, feather pulp collected from bird carcasses has been shown to be useful in dead-bird surveillance when tested by RT-PCR.²²

Standard antibody detection methods include the *hemagglutination inhibition* (HAI) test and *plaque reduction neutralization test* (PRNT). The HAI test is hindered by nonspecific reactivity, whereas the PRNT is more specific and may differentiate between antibody reactivity from closely related viruses.³⁰ In the U.S. the PRNT requires biosafety level 3 facilities,

which may limit its use in many laboratories. IgM-capture *enzyme-linked immunosorbent assays* (ELISAs) have been developed for humans, equines, canines, and chickens and are useful for determining recent exposure to the virus.³⁰ The IgM ELISA may be used as a screening test, with PRNT performed to differentiate between St. Louis encephalitis and WNV as confirmation. A blocking ELISA was developed for broad species use and is used to determine the origin of antibody reactivity. A broadly reactive IgG-capture ELISA for bird serum has proved to be effective in a wide variety of birds but needs to be confirmed by PRNT.^{29,30} Antibody persistence in naturally and experimentally exposed birds is variable. In a study of wild-caught rock pigeons (*Columba livia*) naturally infected with WNV, antibodies were found to persist for longer than 15 months, as detected by ELISA and PRNT; maternal antibodies persisted for an average of 27 days. Both tests outperformed the HAI test.²⁹

HOST SUSCEPTIBILITY AND CLINICAL PRESENTATION

WNV has an extremely broad host range, replicating in birds, reptiles, amphibians, mammals, mosquitoes, and ticks.³⁰ Reviews of pathologic findings in various animal species are available.*

Equine

Cases of WNV disease in horses have been documented either by virus isolation or by detection of WNV-neutralizing antibodies every year since 1999. WNV infection in horses and other domestic equids ranges from asymptomatic to fatal encephalitis. Common clinical signs include ataxia, incoordination, lethargy, weakness, hind limb paresis, muscle tremors and fasciculations, recumbency, and death. Experimental studies suggest that about 10% of infected horses develop clinical illness.^{41,61} From 20% to 40% of equine WNV cases result in death or euthanasia.^{69,70,79,80} Horses most likely become infected by the bite of infectious mosquitoes. In a review of 569 cases, the risk of death among nonvaccinated horses was 3 to 16 times higher than in vaccinated horses after one or two doses.⁷⁰

*References 28, 30, 39, 41, 71, 83.

*References 5, 16, 18, 21, 26, 28, 32, 38, 39, 46, 47, 50, 53, 55, 60-62, 67, 69, 71, 75, 80, 81, 82, 83.

Avian

From 1999 to 2005, 284 bird species were reported to the CDC's WNV avian mortality database. Unlike in traditional endemic areas, infected birds in North America have a spectrum of clinical outcomes ranging from no disease to death. Because birds are the natural reservoir species, they may act as dead-end hosts or viral amplifiers. Komar et al.⁴² showed that Passeriformes and Charadriiformes such as the blue jay (*C. cristata*), common grackle (*Quiscalus quiscula*), house finch (*Carpodacus mexicanus*), American crow (*Corvus brachyrhynchos*), and house sparrow (*Passer domesticus*) are all competent reservoirs. Reisen et al.⁶⁸ recently showed that Western scrub jays (*Aphelocoma coerulescens*), house finches (*C. mexicanus*), and house sparrows (*P. domesticus*) have sufficiently high viremia to infect *Culex* mosquitoes.

Some species, especially those of the family Corvidae, order Passeriformes, are highly susceptible.^{40,41,42} Mortality has approached 100% in American crows (*C. brachyrhynchos*) and loggerhead shrikes (*Lanius ludovicianus migrans*), causing potentially severe declines in the overall population in some areas.* Raptors are thought to be extremely susceptible, especially the family Stringidae.^{26,74,82} Seroprevalence in free-ranging raptor species has been documented from 2% to 88%, suggesting wide ranges of exposure and susceptibility in these species.⁷⁴ One captive population in the epicenter of the original outbreak recorded a seroprevalence of 34% in all at-risk birds.^{50,71} A review of five zoologic institutions in Kansas recorded disease in eight species of seven families in the face of emergence in the region.¹⁸ A North American zoologic surveillance system identified serologic evidence in more than 100 species from 2001 to 2005.⁷⁶ In most species, common clinical signs included anorexia, weakness, depression, weight loss, recumbency, and death with no previous clinical signs of infection. Neurologic signs (ataxia, tremors, disorientation, circling, impaired vision, abnormal head posture) have been widely reported in most susceptible species as well.^{26,30,41,50,71} Hematologic abnormalities reported include leukocytosis and heterophilia, with infrequent monocytosis and reactive lymphocytes.^{18,50,71}

Other Species

In addition to equid and avian species, WNV has caused clinical illness and mortality in many other

species. Dogs and cats were found seropositive at 26% and 9%, respectively, in a large outbreak in Louisiana.³⁵ A Maltese terrier succumbed to WNV encephalitis in 2002.⁶⁷ An Arctic wolf (*Canis lupus*) succumbed with clinical signs of vomiting, anorexia, and ataxia before death; virus was demonstrated in the kidneys and cerebrum on necropsy.⁴⁷ Two WNV-infected alpacas (*Lama pacos*) showed signs of encephalitis, one severe and one mild. The severe case was euthanized after disease progressed to lateral recumbency and opisthotonos. The mild case recovered after 150 mL of llama plasma with antibodies against WNV was administered intravenously on the first day clinical signs were observed.⁴⁵ Infection in four reindeer (*Rangifer tarandus*) resulted in encephalomyelitis, representing the first known cases in Cervidae.⁶² A Barbary macaque (*Macaca sylvanus*) at the Toronto Zoo became infected with naturally acquired WNV encephalitis and was euthanized.⁶⁰ A poliomyelitis syndrome was observed in a harbor seal (*Phoca vitulina*).²¹ Small mammals, Eastern fox squirrels (*Sciurus niger*), gray squirrels (*Sciurus carolinensis*), a rabbit (*Oryctolagus cuniculus*), and an Eastern chipmunk (*Tamias striatus*) have all shown clinical signs and mortality associated with WNV infection.^{32,38,43} Morbidity and mortality occurred in farmed alligators (*Alligator* sp.) after being fed infected horse meat and in a captive crocodile monitor (*Veranus salvadori*) in North America with natural infection. Farmed crocodiles in Israel (*Crocodylus niloticus*) were seropositive after natural exposure.^{55,72,77}

Serologic evidence of infection has been found in many other captive and wild species, including black bears (*Ursus americanus*), marine mammals, and numerous genera of captive African and Asian mammals.^{25,50,71,77}

PREVENTION AND CONTROL

Although WNV is most often transmitted by the bite of infected mosquitoes, the virus may also be transmitted through contact with infected animals, their blood, or other tissues. Thus, laboratory, field, and clinical workers who handle tissues or fluids infected with WNV or who perform necropsies are at risk of WNV exposure. These workers include laboratory diagnosticians and technicians, pathologists, researchers, veterinarians and their staff, wildlife rehabilitators, entomologists, ornithologists, wildlife biologists, zoo and aviary curators, health care workers, emergency response and public safety personnel, public health workers, and others in related occupations. To minimize

*References 5, 8, 43, 53, 54, 81, 84.

risk, workers should have appropriate personal protective equipment that provides barrier protection, such as gloves, gowns, masks, and goggles or glasses with solid side shields and chin-length face shields. Personnel must wash hands and other skin surfaces with soap and water immediately after contact with blood or other tissues, after removing gloves, and before leaving the workplace. Proper disposal or decontamination of sharps and other work-related equipment is important.

Arboviral encephalitis may be prevented in three major ways, as appropriate: (1) protective measures to reduce contact between animals/humans and mosquitoes, (2) mosquito control measures to reduce the number of infected vectors in the environment, and (3) vaccination. Early detection of WNV infection is the best prevention. Ideally, proper epidemiological surveillance programs should be in place for all important vectors, reservoirs, and potential amplifying hosts.⁵⁶ Human and animal protection measures in the presence of WNV activity include reducing time outdoors during periods of high mosquito activity, particularly early morning and evening; wearing long pants and long-sleeved shirts; and applying mosquito repellent to exposed skin areas and clothing where appropriate. Public health measures include elimination of larval habitats or spraying of insecticides to kill juvenile (larvae) and adult mosquitoes. In emergency situations, wide-area aerial spraying is used to reduce quickly the number of adult mosquitoes.

Currently, two WNV vaccines are commercially available in the United States. The West Nile-Innovator vaccine (Fort Dodge) was granted full licensure in February 2003. The manufacturer recommends two initial doses of this killed-virus product intramuscularly 3 to 6 weeks apart, then annual booster vaccination. Protection from disease is reportedly achieved about 6 weeks after the second initial vaccine dose.²⁰ A lyophilized WNV vaccine plus a sterile liquid diluent, RecombiTEK (Merial), was released in January 2004 and has been approved for veterinary use by the U.S. Department of Agriculture (USDA). RecombiTEK contains a recombinant canarypox-vectored vaccine that has been modified to express the desired antigens capable of stimulating a protective response to WNV. The manufacturer recommends two initial doses 4 to 6 weeks apart, as well as a single annual booster. West Nile-Innovator and RecombiTEK vaccines work in completely different ways and cannot be used interchangeably.

Because of the high mortality in some species of high monetary or conservation importance, some practitioners have chosen to use the vaccine in an

“extralabel” manner. Although the vaccine seems to be safe, its efficacy is in question. In cases in which vaccination has resulted in a measurable antibody titer, challenge studies were rarely performed, so minimal standardized data exist for extralabel use. Endangered Eastern loggerhead shrikes (*Lanius ludovicianus migrans*) were vaccinated (1 mL, 2 × 0.5 mL, on either side of the pectoral muscle with boosters at 3 and 6 weeks) in a captive breeding facility, and 84% had detectable neutralizing antibodies.⁵ There is also evidence that the vaccine is safe and elicits an antibody response in corvids and raptors,³⁴ while another study showed a lack of response in flamingoes and red-tailed hawks.⁵⁹ One study showed that the vaccine was safe in alpacas and llamas; administration of three vaccinations (1 mL) generally resulted in similar antibody titers as two vaccinations in horses.⁴⁴

Numerous new vaccines are in development, including those using avian models.¹⁵ One clinical trial of note was performed using an experimental deoxyribonucleic acid (DNA) vaccine developed at the CDC. A recombinant DNA plasmid vaccine in an aluminum phosphate adjuvant that protected fish crows (*Corvus ossifragus*) and American crows (*C. branchyrrhynchus*) during a challenge study was used to vaccinate Andean and California condors (*Vultur gryphus*, *Gymnogyps californianus*). No adverse reactions were present, and a positive antibody response was seen.^{73,79}

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CHAPTER 2

Current Diagnostic Methods for Tuberculosis in Zoo Animals

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Tuberculosis (TB) is a cause of significant morbidity and mortality in both domestic and wild animals worldwide. Although a wide variety of mycobacteria are pathogenic in mammals, birds, reptiles, amphibians, and fish, “tuberculosis” refers to infection with specific organisms belonging to the *Mycobacterium tuberculosis* complex. The presence of TB in zoologic collections has been documented for at least 100 years and suspected to affect wildlife species even longer.

The interaction of free-ranging wildlife and domestic livestock in many countries has led to complex disease issues regarding the control of TB. Furthermore, the zoonotic potential of these organisms presents an additional concern for animal handlers and the public. Therefore, rapid, accurate diagnosis in wildlife species is important not only to zoo veterinarians, but also to those responsible for managing wildlife, to regulatory bodies, and to the public.

ETIOLOGY

The TB complex includes *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and *M. pinnipedii*.^{11,14} *M. tuberculosis* is the predominant cause of TB in humans and elephants, whereas *M. bovis* is the most common cause of TB in domestic animals and wild mammals.³⁰ *M. microti* is primarily found in small rodents (voles) and hyraxes but has also been isolated from llamas, pig, and ferrets. *M. africanum* is a rare cause of TB in humans, cattle, and pigs.

Mycobacterial classification has typically relied on biochemical and phenotypic characteristics of the organisms. These bacteria are slow growing and take up to 8 weeks to appear on Löwenstein-Jensen media cultured aerobically at 37°C. Culture morphology varies from coccoid to filamentous, and microscopically the rod-shaped bacteria are 0.2 to 0.6 µm × 1.5 to

–3.0 µm. Presumptive identification of mycobacteria may be made by demonstrating acid-fast staining characteristics using Ziehl-Neelsen or the Kinyoun staining techniques with carbolfuchsin. In addition to biochemical differentiation, deoxyribonucleic acid (DNA)–specific probes have been developed to provide speciation.^{1,39} Strains have also been identified within species using restriction fragment length polymorphism (RFLP) of identified sequences, spoligotyping, and DNA sequencing.^{30,33}

DIAGNOSTIC TESTS

No antemortem test is 100% reliable for detecting TB in zoo animals. The approach to routine screening and clinical examination of suspect cases requires application of multiple testing modalities. It is important to realize that most tests are not validated in zoo animal species, and those based on immunologic responses especially may show significant variability among species. As technology and knowledge expand, the ability to interpret these tests will increase, but until then the clinician using these diagnostic methods is advised to use caution and understand the potential limitations of each test. A brief synopsis of current diagnostic test modalities follows; the reader is advised to refer to more extensive literature reviews on the subject.

Testing Based on Detection of Mycobacterial Organisms

Diagnostic tests that identify the mycobacterial organism, or components, are the most definitive method of detecting infection. Culture and speciation is considered the “gold standard” and also takes the longest to obtain results (up to 8 weeks, or more for

speciation). Even in human cases, infection is only demonstrated in 50% of adult cases by proof of bacilli in biologic samples.³⁸ Site of infection, intermittent shedding, and difficulty of obtaining samples from some species may lead to decreased recovery of organisms. Laboratories with expertise in mycobacterial culture should be chosen when submitting samples. If treatment is being considered in highly valuable or endangered individuals, culture is necessary for identification and antibiotic sensitivity testing. Improved culture methods, such as at BACTEC, Septi-Chek, MB/BacT systems, and mycobacterial growth indicator tubes (MGITs), have the potential to decrease time to detection of growth and increase rate of recovery.³¹

Direct staining of sample material may provide presumptive identification as acid-fast bacteria, but there are also nonmycobacterial organisms, such as *Nocardia*, that may stain positive. Immunohistochemical staining of tissues is also useful for antemortem diagnosis in limited cases in which biopsy or other relevant samples (e.g., lymph node) may be available. Labeled monoclonal antibodies may confirm acid-fast organisms in tissues as being mycobacteria.

Amplified *M. tuberculosis* direct test (MTD) and multiplex polymerase chain reaction (PCR) assays may provide rapid results by detecting nucleic acid from the organism in clinical samples.^{33,39} Gene probes are used for rapid identification of mycobacterial isolates, whereas the gene amplification methods such as PCR are used to aid in identification of species as well as to test culture-negative samples.^{30,39} By choosing the appropriate primers, PCR tests may distinguish between *M. tuberculosis* complex and *M. avium*.

PCR may also be performed on postmortem samples, including formalin-fixed tissues.³⁹ A combination of techniques was compared for postmortem detection of *M. bovis* in white-tailed deer (*Odocoileus virginianus*). Histopathology had a positive predictive value (PPV) of 94%, acid-fast staining had a PPV of 99%, and application of an *M. tuberculosis* group-specific genetic probe had 100% PPV compared with mycobacterial culture.²⁰

Secreted antigens from proliferating mycobacteria have been the focus of recent diagnostic research. *Antigen 85* (Ag85), produced during active infection, has been detected in sera using dot blot immunoassay. Nyala (*Tragelaphus angasi*) with pulmonary granulomatous lesions had elevated values of Ag85 compared to those with no history of exposure to *M. bovis*.³⁶ However, similar tests on orangutans showed equivocal results.³² Serum Ag85 could be used as an adjunct test but appears to require further validation in each species.

Testing Based on Immunologic Response to Mycobacteria

Cell-Mediated Immunologic Tests

The most common diagnostic test for TB in mammals is the intradermal test, based on in vivo, delayed-type hypersensitivity response to tuberculin antigens. Purified protein derivative (PPD) tuberculins prepared from *M. bovis* and *M. avium* are used for single and comparative testing, particularly of ungulate species.³⁰ The standard dose is 0.1 mL (5000 tuberculin units) in mammals, injected intradermally, usually in the caudal tail fold, skin of the cervical region, or upper eyelid of primates. Other sites used include the lateral thorax, axillary region, abdomen, and ear. Old tuberculin (OT), prepared from either *M. tuberculosis* or *M. bovis*, has historically been used in primates and zoo ungulates but has been phased out because it is more difficult to standardize between lots and is less specific. Currently, most PPD tuberculin is produced at a protein concentration of 1 mg/mL.³⁰ Ideally, injection sites are measured with calipers at initial injection and again after 48 hours in nonhuman primates and swine or after 72 hours in ungulates. Specific criteria for “negative” and “suspect” have been developed only for a few nondomestic species, including some cervids. If swelling is present, additional diagnostic testing, including a *comparative cervical test* (CCT), is warranted. Ancillary tests, such as the interferon-gamma (IFN- γ) test, have been approved in the U.S. federal eradication program for domestic cattle to replace or augment the results of CCT. The basis of the CCT is that there will be a differential response to *M. avium* and *M. bovis* PPD based on whether the animal is infected with *M. tuberculosis* complex or has had a transitory sensitization from nontuberculous mycobacteria.

Intradermal testing is fraught with problems, including anergic responses in individuals with fulminant disease, species and individual variability in response, and false-positive and false-negative reactions. Even in humans, the positive predictive value for tuberculin skin test varies with infection prevalence in the tested population, with at least a PPV greater than 75% in which infection prevalence was above 10%, but decreased PPV in populations with lower prevalence.³ Certain zoo species are known to have an increased likelihood of nonspecific reactions, including tapirs, bongo antelope, reindeer, and orangutans. To address these issues, the use of purified antigens in vivo and in vitro is being investigated in a variety of species.

Diagnostic tests based on in vitro cell-mediated immune responses to mycobacteria include lymphocyte transformation, cytokine production (i.e., IFN- γ , interleukin-2), and other indirect measures of immunologic stimulation, such as cytokine ribonucleic acid (RNA) assays. *Lymphocyte transformation* (LT) tests are performed by stimulating mononuclear cells with specific antigens and then incubating the proliferating cells with a radioisotope-labeled nucleotide. The amount of label incorporated is correlated with the degree of proliferation and is an indicator of previous exposure and immune recognition of the specific antigen. The LT assay was part of the blood tuberculosis (BTb) test developed to overcome the problems associated with skin testing and was used as an ancillary test for U.S. deer in the 1990s.¹² A similar comparative lymphocyte stimulation test developed for *M. bovis*-infected Eurasian badgers (*Meles meles*) using bovine and avian tuberculin showed 87.5% sensitivity and 84.6% specificity.¹⁷

Assays that measure cytokine production, such as IFN- γ and interleukin-2 (IL-2), appear to be more sensitive than skin tests. Cytokines are generally more conserved between species, so detection methods may be more widely applicable. For example, the immunoassay developed for human IFN- γ was able to detect chimpanzee, orangutan, gibbon, and squirrel monkey IFN- γ and correlated with in vivo tuberculin skin reactivity.¹⁹ This test was commercially available as Primagam (CSL Veterinary, Australia) for use in gorilla, orangutan, chimpanzee, gibbon, guereza, mandrill, squirrel monkey, marmoset, and baboon. A similar assay was produced for cattle (Bovigam), deer (Cervigam), and humans (Quantiferon). The IFN- γ test has been used with African buffaloes (*Syncerus caffer*) to aid in a test and cull program for bovine TB in Kruger National Park, South Africa.²⁴ Necropsy and culture results were used to confirm field cases, and the specificity of the IFN- γ test was shown to be 99.3%. Recent research investigating other cytokine production (e.g., IL-2) or cytokine RNA may provide additional in vitro methods of assessing response to mycobacterial infection across a range of species.⁴⁴ Difficulties associated with using these assays include (1) specific culture parameters need to be developed for each species, and (2) whole blood needs to be properly handled for accurate test results. Many of these tests are not currently available on a commercial basis.

Serologic Tests

Enzyme-linked immunosorbent assay (ELISA) has been the most frequently used serologic test for TB diag-

nosis. These assays incorporate various forms of mycobacterial antigens for detection of antibodies in the test sample and also are a component of the BTb test. In one study of 12 cervid herds, the specificity and sensitivity of a five-antigen ELISA were 78.6% and 70.0%, respectively.²¹ The ability to diagnose TB increased if ELISA and tuberculin skin test results were used in parallel, rather than using either test alone.

ELISA has been used to evaluate *M. bovis* infection in brushtail possums (*Trichosurus vulpecula*) in field tests.⁶ The sensitivity and specificity of the assay using *M. bovis* culture filtrate was 45% and 96%, respectively, and the results were 21% and 98% when the antigen was MPB70. Further study showed that *M. bovis*-infected possums develop antibody late in the course of disease that may affect the sensitivity of serologic diagnostic tests for this species. This underscores the importance of understanding the immunologic response to TB in each species and the potential limitations of serologic assays.

With the development of purified, recombinant, and fusion proteins, tailored antigen panels may be developed to change specificity and sensitivity of serologic tests. In addition, other methods may be employed, such as Western blot (immunoblot), thin-layer immunochromatography, and multiantigen print immunoassay (MAPIA). Immunoblot has been demonstrated to be a sensitive method to detect and monitor development of serologic response to specific mycobacterial protein antigens in a variety of species.⁴⁹ Immunodominant antigens may be identified and used for development in other serologic assays, such as ELISA or immunoblot. MAPIA entails application of antigens to nitrocellulose membranes, followed by incubation with test sera and detection using standard chromogenic immunodevelopment.³⁵ MAPIA has been useful in choosing antigens appropriate for a rapid test that utilizes thin-layer immunochromatography and may provide a diagnostic screening test for field situations.²³

In a study comparing serologic and cell-mediated responses to *M. bovis* in reindeer, antibody could be detected as early as 4 weeks after experimental infection.⁴⁹ Animals tested positive using multiple serologic tests but showed individual variation in antigen recognition at different time points. MAPIA appeared to be most sensitive and detected antibodies earliest after infection at 4 weeks, immunoblot at 8 weeks, and ELISA at 15 weeks. When compared with IFN- γ and skin test responses, all the infected reindeer tested positive by CCT at 3 and 8 months after infection, but no correlation was found between skin test reaction

and level of antibody. Similarly, there was no correlation between antibody levels and IFN- γ response. This study shows the potential diagnostic value of serologic tests in a species that has a low prevalence of disease and a high number of nonspecific reactions with skin testing.

CURRENT PROTOCOLS FOR ZOO ANIMALS

Tuberculosis, caused by *M. bovis* or *M. tuberculosis*, is a reportable disease in the United States. Worldwide, TB is one of the infectious diseases that causes the greatest annual morbidity and mortality in humans, with an estimated 2 to 3 million deaths each year.³⁰ TB has been diagnosed in most mammalian taxa typically housed in zoologic collections. Sporadic cases, as well as epizootics, have occurred in zoos around the world.^{16,33,46}

The diagnosis of TB in a zoologic collection may lead to restriction of animal movement, issues associated with human health, and euthanasia of potentially healthy animals. To address these concerns, the National Tuberculosis Working Group for Zoo and Wildlife Species was established to develop protocols for testing and movement of zoologic species, with a focus on nondomestic hoofstock and elephants.⁴⁸ The protocol *Guidelines for the Control of Tuberculosis in Elephants* is available on the American Association of Zoo Veterinarians (AAZV) website (www.aazv.org); *Tuberculosis Surveillance Plan for Non-Domestic Hoofstock* is being finalized. Additional goals of the surveillance plan are to establish data on diagnostic methods and estimate the true prevalence and incidence of TB in zoologic collections.

Guidelines for testing primates are often based on standards developed by the World Organization for Animal Health (OIE), Centers for Disease Control and Prevention (CDC), and National Institutes of Health (NIH). Origin, history of close human contact, and environment are primary risk factors in determining likelihood of TB in nonhuman primates. Certain species and exposure to other mycobacteria have been correlated with an increase in false-positive skin reactions.⁹

More recently, the Veterinary Advisory Group of the Animal Health Committee of the Association of Zoos and Aquariums (AHC-AZA) have started to develop taxon-specific or species-specific recommendations for preshipment and preventive health protocols that include standardized diagnostics, such as TB testing. This approach may facilitate data collection for determining the validity of various diagnostic tests for TB.

CLINICAL FINDINGS

Tuberculosis should be on the differential list for any mammal that exhibits clinical signs of chronic weight loss or emaciation, weakness, dyspnea, cough, and enlarged lymph nodes. Unfortunately, many infected animals are asymptomatic until disease is advanced. Therefore, a proactive quarantine and routine screening program should be developed for each zoologic collection housing susceptible species.

Primates

Primates may be infected by *M. bovis*, *M. tuberculosis*, *M. avium*, and rarely, other nontuberculous mycobacteria. It is important that diagnostic tests differentiate pathogenic mycobacterial infections from potential cross-reactions caused by exposure to other nontuberculous mycobacteria. The most common method of screening nonhuman primates is intradermal testing. OIE recommends that all imported prosimians, callitrichids, New and Old World monkeys, gibbons, and great apes be tested at least two or three times at 2- to 4-week intervals during quarantine (OIE Terrestrial Animal Health Code, 2005). Nonhuman primates require 1000 to 10,000 times more tuberculin than humans to elicit a delayed hypersensitivity response.⁹ Therefore, it is important to use products manufactured for nonhuman primates, with a minimum dose of 1500 tuberculin units/0.1 mL. The most common site for injection is the upper eyelid, which is examined visually at 24, 48, and 72 hours for degree of swelling and erythema. Other injection sites include arm, thorax, or abdomen, especially in smaller species such as callitrichids. Because mammalian OT is a nonuniform product that may vary between batches, nonspecific reactions may be observed in uninfected primates. Some newer recommendations have switched from using mammalian OT to mammalian PPD in the single intradermal test because content is more easily standardized in these preparations. Comparative tests using mammalian and avian PPD, along with ancillary tests, should be performed in any individual that has a suspect reaction.

Additional diagnostic tests include complete blood count (CBC); thoracic radiographs; mycobacterial culture (may be done from lesions and tracheal/gastric lavage); PCR/MTD; acid-fast staining of tracheal/gastric lavage, feces, or tissue; and immunoassays. Molecular techniques such as PCR/MTD and RFLP may be used to distinguish pathogenic mycobacterial infections from atypical infections that may cause a

positive tuberculin skin response. This method was used to identify asymptomatic *M. kansasii* infections in several squirrel monkeys that were suspect responders.⁵ In a zoo study of 68 New World primates, different species of mycobacteria were detected by PCR in 65% of the primate population, of which 11% were diagnosed as *M. tuberculosis* by gene amplification and RFLP.¹ Only 54% of this population was culture positive.

Several immunoassays have been used for TB diagnosis in primates. The IFN- γ test (Primagam) uses whole blood and has been tested in gorillas, chimpanzees, orangutans, gibbons, colobids, baboons, mandrills, vervets, guenons, squirrel monkeys, langurs, and marmosets, but it cannot detect IFN produced by cells from *Macaca* spp.⁴

ELISA and MAPIA have also been used to evaluate serologic responses in nonhuman primates. *M. bovis*-infected macaques developed antibodies that were detectable in an ELISA using ESAT-6 as the antigen.²⁹ Although these tests are promising, they are not commercially available at this time.

Routine screening of primate collections depends on the history of the collection and assessment of risk factors, such as exposure to other primates, including humans. Because mycobacterial infections may be insidious, periodic screening is recommended even in closed collections. A thorough necropsy of every nonhuman primate that dies should be performed and mycobacterial culture and PCR of thoracic lymph nodes and other tissues considered even in the absence of gross lesions, if there has been a history of exposure or infection in the group. Tissue should be archived for future analysis if any suspicious lesions are observed.

Carnivores

In general, TB in carnivores occurs only sporadically from incidental infection through close contact with infected reservoir hosts or ingestion of infected animals. *M. bovis* has been detected in lions, cheetahs, domestic dogs and cats, leopards, tiger, red fox, and fennec fox, and *M. tuberculosis* complex in snow leopards and domestic dogs and cats.^{2,25,27}

The intradermal skin test has been used to screen lions antemortem.⁴¹ South African lions in an area with a high prevalence of *M. bovis* were tested using an intradermal CCT.⁷ Positive skin tests showed good correlation with necropsies revealing suspicious lesions and positive cultures. Therefore, it appears that comparative intradermal testing may be modified for use as a screening test in lions and potentially other exotic felids.

Routine tuberculin testing of felids and canids is not standard in most zoologic collections. Imported or wild-caught carnivores from regions that have a known TB reservoir should be screened during quarantine. Additionally, carnivores that are fed carcasses that might harbor organisms (e.g., whole-prey feeding practices) should be evaluated periodically. The diagnostic workup includes CBC; thoracic radiographs; tracheal/gastric lavage, feces, or tissue for acid-fast stain; PCR; and mycobacterial culture. A single or comparative intradermal tuberculin test using bovine and avian PPD may also be used for screening, although response data are extremely limited for most carnivore species. PCR has been useful in rapid detection of organisms and distinguishing between *M. avium* and *M. tuberculosis* complex with appropriate primers. DNA fingerprinting is useful for identification of strains and epidemiologic investigation. A thorough necropsy should be performed on any carnivore that dies and tissues cultured and archived if there is a suspicion of TB.

Immunoassays have also been used to a limited degree in carnivores. Serum from a *M. bovis*-infected lion was positive in ELISA to *M. bovis* antigens, whereas tuberculin test-negative cage mates were ELISA negative.⁴¹ ELISA has also been used to screen East African lions.¹⁰ Recently, sera from a group of *M. bovis*-infected jaguars were tested using Rapid Test and MAPIA.³⁴ Serologic results were consistent with culture status. IFN- γ tests, similar to Primagam, have not been developed for carnivores to date.

Small Mammals

Tuberculosis has been diagnosed in ferrets, hedgehogs, badger, voles, hyrax, rabbit and hare, stoats (*Mustela erminea*), mole (*Talpa europaea*), and brown rat and reproduced experimentally in mice, rabbits, and guinea pigs.^{15,18} The primary focus of testing has been identification of wildlife reservoirs for management and control. Most cases are diagnosed postmortem based on gross lesions, histopathology, culture, and PCR identification of the mycobacterial organism, usually *M. bovis*. Immunoassays detecting cell-mediated responses and antibody have been investigated in *M. bovis*-infected badgers.^{23,44} Although not routinely screened, a case of TB caused by *M. microti* in an imported hyrax emphasizes the need for surveillance and the lack of available tests for TB detection in these species.¹⁵

The diagnostic workup for any suspect case includes CBC; thoracic or whole-body radiographs;

tracheal/gastric lavage, feces, or tissue for acid-fast stain and PCR/MTD; and mycobacterial culture with speciation. Intradermal tuberculin test has not been evaluated in the majority of these species. DNA fingerprinting should be performed when possible to determine relatedness of isolates and origin when more than one case is involved.

Marsupials

Mycobacterial infections are important diseases of marsupials, although *M. bovis* has been found primarily in the brushtail possum.¹¹ *M. avium* and other atypical mycobacteria are a greater concern for other marsupials, such as tree kangaroos and wallabies.²⁸ These infections usually present as osteomyelitis. *M. bovis* and *M. tuberculosis* may also cause osteomyelitis, so it is important to be able to distinguish between these infections.

Tuberculin testing of marsupials has not been standardized. It appears that differences in cell-mediated immune response may play a role in the preponderance of primarily *M. avium* infections observed in this group of mammals.⁴⁰ Positive intradermal tuberculin tests to *M. avium* have been observed in infected tree kangaroos.²⁸ Diagnostic examinations should include CBC, chemistry panel, whole-body radiographs that include the skeletal structures, acid-fast stain, mycobacterial culture, and PCR on exudates from draining tracts, lymph node, or other biopsy samples (bone). ELISAs were evaluated in possums but had insufficient sensitivity for widespread application in field situations.⁶ Molecular techniques, such as PCR and DNA fingerprinting, may be used to distinguish among the various mycobacterial species, which is important from a regulatory, zoonotic disease potential, and disease management perspective.

Routine evaluation of marsupials for mycobacterial infection is not typically performed except in quarantine or wildlife screening programs. If marsupials are being examined for other reasons (e.g., routine or preshipment exam), an assessment to rule out asymptomatic infection should be included.

Marine Mammals

Marine mammals are susceptible to infection with a variety of mycobacterial species. Tuberculosis has been found in both captive and wild pinnipeds, caused by a unique member of the *M. tuberculosis*

complex, *Mycobacterium pinnipedii*.¹⁴ This organism is also pathogenic in guinea pigs, rabbits, humans, and Brazilian tapirs. Clinical signs include depression, lethargy, dyspnea, and weight loss. Asymptomatic infection and acute mortality may occur in affected populations.

Diagnosis of TB in pinnipeds usually includes CBC, chemistry panel, ELISA using mycobacterial antigens, thoracic radiographs, acid-fast stain, mycobacterial culture, and PCR of respiratory or other exudates/tissue, and intradermal tuberculin tests. Tuberculin tests using bovine and avian PPD have been assessed in several species of pinnipeds.⁴² Of 40 animals tested, 14 reacted positively to both tuberculins. Ten (of 14) responders had gross lesions at necropsy and/or positive cultures. ELISA results using *M. bovis* antigen also appears to correlate with mycobacterial infection, although it is unknown how exposure to nontuberculous mycobacteria may affect results.¹³

Routine TB testing is not usually performed in pinnipeds. Because *M. pinnipedii* apparently may be brought into a collection with wild-caught animals, however, screening in quarantine and periodic opportunistic testing should be considered as part of the preventive veterinary medical program.

Ungulates (Bovids, Giraffe)

Tuberculosis in artiodactylids is usually caused by *M. bovis* but has also been associated with *M. tuberculosis* infections. Although the U.S. federal eradication program only requires testing of cattle, bison, and cervids, the disease is reportable in all species. The *caudal fold tuberculin test* (CFT) is the official test for routine use in cattle and bison. The CFT is performed by injecting 0.1 mL of bovine PPD tuberculin (1 mg/mL) intradermally in the tail skin fold, with reading by visual observation and palpation at 72 (\pm 6) hours. The *comparative cervical tuberculin test* (CCT) is the official method for retesting suspects. The bovine IFN- γ assay may be used as an alternative method for retesting cattle herds, with appropriate approval (USDA APHIS Bovine TB Eradication Uniform Method & Rules, 2005). Histopathology, mycobacterial culture, and PCR are also approved supplemental diagnostic procedures.

Among exotic species, TB has been recorded in greater and lesser kudu, common duiker, African buffalo, lechwe, eland, impala, American bison, water buffalo, Arabian oryx, East African oryx (*Oryx gazelle beisa*), wildebeest, topi, bushbuck, goats, sheep,

mountain goat, addax, sable antelope, and giraffe, although all cloven-hoofed ungulates are considered susceptible.^{2,10,11,16} Surveys of tuberculin testing in zoo hoofstock have indicated variability in types of tuberculin used, site of injection, and interpretation of tests.^{45,50} The National Tuberculosis Working Group for Zoo and Wildlife Species has developed standardized recommendations for intradermal testing in exotic ungulates. For program species (bison, domestic cattle) and *Bos*, *Bubalus*, and *Snycerus* bovids, the recommended test site is the caudal tail fold. The *single cervical tuberculin test* (SCT) is recommended for all other exotic bovids using 0.1 mL of bovine PPD, read at 72 hours. TB testing in giraffe is usually performed by CFT or SCT. Unless there is a history of TB in the herd or suspicion of infection based on clinical signs, immobilization for routine screening of giraffe is not recommended.

Because of variable sensitivity and specificity of intradermal testing in exotic hoofstock, other diagnostic tests should also be used, especially if an animal has a suspected infection. ELISA has been used in a limited number of species and may aid diagnosis in anergic individuals.¹⁶ Nasal swab, tracheal/bronchial lavage, or material from draining lymph nodes or other tissue may be sent to the laboratory for mycobacterial culture, acid-fast stain, and PCR/MTD.

Immunoassays have been adapted for use in exotic ungulates, but development is often hindered by the need to develop species-specific test parameters or reagents. The IFN- γ assay and LT test are both experimental and have been used in a limited number of exotic hoofstock species, such as American bison and African buffalo.^{2,24} Rapid Test and MAPIA were positive in an *M. tuberculosis*-infected Addra gazelle.³⁴ It appears that serologic tests may be useful as ancillary tests in some species.

Because of the possibility of TB in exotic bovids and regulatory concerns, it is recommended that zoo ungulates undergo screening during quarantine. Frequency of routine testing of collection hoofstock will depend on relative risk and factors such as potential exposure to infected animals, both inside the collection and outside (i.e., wildlife reservoirs), herd history, management practices, and environment. Similar to the requirements for domestic cattle herd accreditation, after initial screening of the herd, it would be prudent to screen adult animals every 2 years, or as the opportunity arises, because immobilization or handling may not be warranted in some situations. All hoofstock that die or are euthanized should receive a complete necropsy, especially focusing on the cervical and

thoracic lymph nodes and respiratory system, to rule out TB.

Cervids

Cervid TB is an important disease in captive and free-ranging populations worldwide. *M. bovis* has been found in a wide variety of species, including elk, white-tailed deer, sika deer, reindeer, mule deer, fallow deer, and moose, although *M. tuberculosis* and *M. avium* have also been isolated.¹¹ Because of potential zoonotic and agricultural impacts, cervid TB is a federally regulated program in the United States.¹² Interstate movement of cervids in the United States requires TB testing of the cervids. States may adopt more stringent requirements regarding intrastate movement. AZA-accredited facilities are exempt from some of the rules when moving cervids between member facilities. These regulations are subject to change and should be checked before transport.

Currently, the SCT is the primary diagnostic test used in captive cervid herds with animals older than 1 year (USDA APHIS Bovine TB Eradication UMR, 1999). The test is performed by intradermal injection of 0.1 mL of bovine PPD tuberculin (1 mg/mL) in the midcervical region, with reading by visual observation and palpation at 72 (± 6) hours. The CCT is used for retesting SCT suspects and is administered by a state or federal veterinarian. Histopathology, mycobacterial culture, and PCR are supplemental diagnostic procedures approved in the federal program. Results of all approved tests must be submitted to state and federal animal health officials.

Because of variable specificity and sensitivity of these tests and the difficulty distinguishing *M. bovis* infections from those caused by *M. avium* and other mycobacteria, alternate diagnostic tests should also be performed in suspect cases.¹² The BTb test, a combination of ELISA and LT assay, is no longer available in the United States as a commercial assay but has been replaced with an IFN- γ assay, Cervigam. This may be used as an ancillary test to CCT. Other diagnostic tests include lymphocyte stimulation tests, ELISA, immunoblot, Rapid Test, and MAPIA.^{11,12} These have been especially helpful in species such as reindeer in which the low prevalence of TB and high frequency of false-positive tuberculin reactions have led to difficulty with diagnosis.⁴⁹

A sound preventive medicine program should include regular TB testing of cervids in the zoologic collection. Incoming cervids should be tested before

transport and/or before leaving quarantine by tuberculin skin test and at least one ancillary test method, if available; otherwise, serum should be banked. Frequency of routine screening of cervid herds will depend on herd and collection history of TB exposure, type of herd management (closed or regular new additions), exposure to other potential sources of infection (e.g., mixed-species exhibits), and risk of handling for testing. Because the federal program requires an accredited TB-free cervid herd to pass two repeat herd tests every 2 to 3 years, screening of zoo cervids at the same frequency would be reasonable, using a combination of SCT and available blood-based tests.

Any cervid showing clinical signs consistent with *M. bovis* infection should receive a thorough examination, including CBC, chemistry panel, thoracic radiographs, and SCT; tracheal/bronchial lavage for acid-fast stain, mycobacterial culture, and PCR/MTD; possible lymph node aspirate or biopsy for histopathology and culture, PCR, and acid-fast stain; and blood collected for immunoassays, if available (IFN- γ production, ELISA, Rapid Test, MAPIA, Ag85). Complete necropsy should be performed on a cervid that dies or is euthanized, with special emphasis on head, cervical, thoracic lymph nodes, and respiratory system.

Camelids

Tuberculosis is found in both New World and Old World camelids. Routine screening is recommended as part of their regular health evaluation and may be required by regulatory agencies for interstate or international movement. Intradermal testing is usually performed by clipping hair in the postaxillary region and injecting 0.1 mL (5000 tuberculin units) of bovine PPD tuberculin. Skin thickness is measured at injection and 72 hours later, and any increase greater than 1.0 mm is interpreted as a response (USDA APHIS VS National Center for Import and Export). Responders should be retested by CCT. Additional diagnostic testing may include thoracic radiographs in smaller individuals; mycobacterial culture, acid-fast stain, and PCR of tracheal/bronchial wash or other fluids/tissues; and immunoassays, if available. Although bacille Calmette-Guérin (BCG)-vaccinated alpacas showed some response in LT and ELISA, experimentally *M. bovis*-infected llamas did not demonstrate a positive serologic response.^{26,47} In naturally infected Bactrian camels, ELISA and immunoelectrophoresis detected antibodies to multiple

mycobacterial species, including *M. bovis*, which may explain why camelids show a high frequency of false-positive tuberculin reactions.⁸ Rapid Test has shown promise in diagnosing naturally infected Old World camels.³⁴

Camelids should be screened regularly for TB as part of a thorough preventive health program, including quarantine and preshipment evaluation. Frequency of screening can be determined based on ease of handling, history of the individual, herd, and collection.

Tapirs

Pulmonary infection with *M. bovis* and *M. tuberculosis* has been reported in captive tapirs.⁴⁵ Regular screening is recommended. Bovine PPD tuberculin (0.1 mL) should be injected in the inguinal region near the nipples, although the skin around the perineum may also be used. Similar to camelids, tapirs may show nonspecific reaction to intradermal testing, confounding interpretation. Another recommended method of diagnostic screening is to flush 20 mL of sterile saline in one nostril, then collecting the rinse by gravity or aspiration in a vial for mycobacterial culture and PCR.⁴³ Immunoassays developed for other species, such as LT and ELISA, have been evaluated on a limited basis in tapirs, but may not be available.

Rhinoceroses

Tuberculosis has been diagnosed in captive black and white rhinoceroses.^{33,37,46} Both *M. tuberculosis* and *M. bovis* have been isolated from black rhinoceroses. Intradermal testing using 0.1 mL of bovine PPD injected in the eyelid, base of the ear, or caudal tail fold has been used for screening rhinoceroses.²² If present, swelling should be followed by immobilization to collect tracheal lavage for acid-fast stain, mycobacterial culture, and PCR for identification.³³ Serologic tests, such as ELISA, Rapid Test, and MAPIA, are also being investigated in these species.

With the increased use of husbandry training and restraint chutes for rhinoceroses, health screening may be accomplished on a more regular basis. Tuberculin testing and serologic screening should be incorporated into the preventive health program for these species based on history of the herd and collection. All rhinoceroses should ideally be screened as part of a thorough preshipment and quarantine evaluation.

Elephants

See Chapter 43 for a discussion of tuberculosis in elephants.

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CHAPTER 3

Infrared Thermography in Zoo and Wild Animals

SABINE HILSBURG-MERZ

Infrared (IR) thermography is a noninvasive diagnostic screening tool that does not require handling or restraint of an animal. Physiologic or pathologic processes involving changes in surface temperature may be evaluated using this technique. This modern method provides real-time, instantaneous visual images with measurements of surface temperatures over a greater distance.

The first medical application of “thermography” was by Hippocrates (ca. 460–375 BC), who used thin layers of mud for his temperature measurements, similar to modern thermography. An area of great heat emission caused an area of the mud to dry first, and thus a “hot spot” was detected.²⁹ It was not until the mid-eighteenth century, however, that temperature scales were developed by Fahrenheit, Réaumur, and Celsius, and not until 1800 that Sir William Herschel discovered infrared rays distinguishable from visible light. The first detector was constructed in 1830.⁶

Infrared thermography has been used for skin temperature measurement in human medicine since 1960 and for the early detection of diseases since 1980, mainly pathologic processes such as pain in the lumbosacral region, intervertebral disc prolapse, spinal cord lesion, traumatic lesions, fractures, neuropathology, cardiovascular diseases (especially impairment of blood supply), lateral effects of heat or frost burns, and long-term monitoring of skin transplants. In wildlife biology, IR thermography has been used since the mid-1940s for detecting and monitoring mammal and bird species. To some degree the method could even be used successfully in animal censuses. In veterinary medicine this technique has been used on farm and companion animals since the late 1950s.⁹ The most advanced field is that of equine medicine.^{24,28,29} Eulenberger and Kämpfer³ first recommended the use of IR thermography in zoo and wild animal medicine.

Phillips¹⁵ performed the first large-scale comparative studies on thermoregulation in zoo animals with

the aid of infrared thermography. Both studies employed traditional, carbon dioxide (CO₂)-cooled systems, which proved to be difficult to use under routine zoo and wildlife conditions. Hilsberg⁹ first used IR thermography extensively with modern equipment in zoo medicine.

METHOD

Infrared thermography makes use of the physical characteristic of bodies or materials to emit electromagnetic waves, and with the aid of a special detector, these rays are visible. Therefore, surface temperatures are measured over a greater distance.⁶

The advantages of IR thermography compared with other imaging techniques (e.g., ultrasonography, radiography, magnetic resonance imaging, endoscopy) are as follows:

1. Is completely noninvasive because no contact with the animal is necessary, and therefore no animal training, immobilization, or sedation is required.
2. Offers an ideal, instantaneous first screening method to help the veterinarian in decision making, monitoring, and determining whether other measures need to be taken.
3. Yields real-time visual imaging in gray or false-color coding.
4. Provides surface temperature imaging of a whole animal, or parts of the animal, as well as easy comparison with herd mates at the same time.
5. Permits examination of motion and direction (e.g., inflammation, reproductive evaluation).
6. Allows easy monitoring of a condition over time (e.g., lameness, inflammation, pregnancy).
7. Facilitates documentation and preservation of primary data.
8. Is portable and uses battery packs and thus is conducive to zoo and wildlife field conditions.

As with other techniques, however, IR thermography presents specific challenges in zoo and wildlife medicine that are not encountered as often in human medicine and classic veterinary medicine. For example, detailed knowledge of the morphology of many different species is required; no control exists over the animal under investigation (e.g., movement, position relative to the sun, muddy or wet surface parts, positioning of animal for best investigation); and no specific examination room in a veterinary clinic with controlled environmental parameters (e.g., temperature) is available.

TECHNIQUE

Using an IR camera or scanner, the heat emitted by every material or object may be detected and made visible through conversion into temperature-associated shades of gray. The warmer areas are colored white or light gray, and the cooler areas are darker gray or black. The system may also use several scales of false-color coding. This means that an image is created in which each temperature is assigned a specific color on a reference scale; the best scale for veterinary diagnostics is the rainbow color scale. The image created can be interpreted and used for diagnostic purposes in medical fields.

The IR camera works similar to a digital video camera, except the lenses possess specific attributes. Because glass hinders the transmission of heat waves, other materials are used as semiconductors, such as germanium-zinc, lead-selenium, or cadmium-mercury-telluride. Each specific mixture of half-metals measures a defined wavelength within the IR spectrum. Each of these wavelength windows possesses specific properties, but also disadvantages, so the industry has tried to optimize the materials used for the required purposes. Gaussorgues⁶ provides detailed information on the physics behind these systems, with a shorter, more veterinary-oriented version by Hilsberg.⁹ Before obtaining a system, the clinician must consider the lens specification.

An IR system should be certified by the regional authorities. Only such systems guarantee that the measured temperatures are accurate and that it is legal to use the system; specific regulations exist because of the military use of this technology. Recently, increasing numbers of systems are appearing on the market that are remakes or copies of earlier units. These systems, however, may not be certified and thus may yield false temperature readings. The potential thermographer should consult with engineers or local experts before

acquiring such equipment. All the studies described by Hilsberg⁹ have used the IR systems by the companies AGEMA and later FLIR Systems. With the enormous technical developments achieved in the last decade, this technique should be used throughout veterinary medicine, especially in zoo and wild animal medicine, as an aid in primary diagnostics.

The images captured by the IR detector may be saved and stored on a hard disc or other storage media and viewed and evaluated later on the computer with specialized software. Each false color or gray point in the image is still associated with the originally measured temperature, so the settings of each image may be optimized for evaluation on the computer.

When using this technique, it is important that investigators are aware of the influences on their readings. The animal should be acclimatized to the environment, preferably for 2 hours before thermal imaging. Furthermore, the animal should be clean, dry, and free of dirt; otherwise, artifacts may be created, which may require interpretation. Under certain circumstances it is more advantageous to have the animal wet and not acclimatized, as explained later under the species-specific investigation techniques.

ANIMALS AND ENVIRONMENT

Thermography is best used on animals, or parts of them, without long hair, such as elephants, rhinoceroses, hippopotami, giraffes, zebras/horses, and many larger antelopes. In longer-haired animals such as carnivores, camels with winter coats, and mountain animals, the interpretation of results is more difficult. In these cases the procedure is better done by an experienced thermographer, unless only joints, feet, or parts of the head are evaluated, although even these may create problems. The thermographer must be familiar with the normal skin surface, internal anatomy, and morphology of the animal under investigation. Regional hair length is an important factor for interpretation, as well as the location of blood vessels and the innervation of skin areas under investigation.

SOURCES OF ARTIFACTS

Clipped hair may increase temperature readings. Alcoholic ointments or other surface heat-producing materials also create artifacts in the form of increased heat emission. On the other hand, cold water, dirt, or mud may create an altered heat emission that shows lower temperatures, at least when first applied. Later,

this foreign material emits the heat according to its composition. Additionally, uneven pelage creates uneven heat transmission. Strong physical activity of the animal will create local heat production at first, but heat emission from the whole-animal surface may occur later, depending on the type of animal and the type and duration of the activity.

High ambient temperature poses difficulties when looking for smaller temperature differences. Under high ambient temperatures the difference between the animal core and surface temperature decreases. This makes the use of IR thermography more challenging in field investigations than in zoo settings. A good way to address this problem is using the technique in a stable or, for wildlife at night, near a waterhole. The sun itself also creates significant artifacts, and therefore cloudy days are preferred. However, clouds still allow a certain quantity of infrared emission. The effect of the sun is especially visible in giraffes and zebras. In zebras the author found specific skin pattern-related heat radiation when the animals were in their stables at night.¹

A brief introduction to these investigations is provided in the later discussion on thermoregulation. Again, the best place for an investigation of a zoo animal is the stable, or the investigations should take place on a cloudy day, after sunset, or before sunrise, if absolute temperatures are required. Otherwise, the investigator should try to lure the animal into a shady part of the enclosure. An experienced thermographer can cope with many artifacts or will do a follow-up investigation a few hours or days later. Artifacts may also result from sources of heat in the housing environment of zoo animals, such as heaters on walls, floor heating, or even heating from ceilings. If not accounted for, these sources may lead to gross misinterpretations, as in pregnancy diagnosis.

OPTIMAL SETTING

When starting to use IR thermography, as just discussed, the best time and place to investigate an animal is the animal's stable early in the morning. This animal is most likely acclimatized, dry, dirt free, and not stressed or physically exhausted. The investigator should look for signs of scratching on the skin. If the stable has floor heating, the animal must be allowed to stand for at least 1 to 2 hours to prevent false readings from that heat source. If the animal is dirty, hosing it down with medium-temperature to cool water may help. The thermographer can then follow up on the process of warming the skin to look for hot areas. This

method is sometimes the best way of investigating elephants and hippopotami.

GENERAL FIELDS OF USE

Thermoregulation: the Basics for Medical Thermography

Before veterinarians can make good use of IR thermography in zoo and wildlife medicine, they must become familiar with the thermoregulatory patterns of each species. This is important because each species presents specific challenges for thermography: color patterns; hair length; thickness of the dermis; location of glands; size of ears, horns, or antlers; location of potential thermal windows on the body itself; and the anatomy of the legs. *Thermal windows* are areas of increased heat emission; some are facultative and some obligatory (see later discussion).

Because of the lack of hair, elephants (and most rhino species) display a relatively even surface temperature under normal conditions, with only the ears, horns, or tusks showing lesser heat radiation than the body and legs. Mammals with short hair and thin legs (e.g., giraffes, antelopes, zebras) display cooler legs than bodies under normal thermoregulatory conditions and in the shade. Animals with thick hair may display little radiation through the body surface, which may make the use of IR thermography almost impossible. However, some uses may still be possible, such as the diagnosis of inflammatory processes on the legs. The inside of mammalian legs shows a slightly greater heat radiation than the outside because of the more superficial location of blood vessels. When doing close-up views of the ears in both African and Asian elephants, the blood vessels may be located easily. Apart from the blood vessels, ears should display no other source of higher radiation, except at the opening of the ear canal. As an example, normal thermoregulation in elephants is judged by viewing cooler ears than body temperature, as well as measuring the overall average body and leg surface temperatures, which should be relatively constant within a limit of about 1° to 2° C.

The only exceptions from this uniform surface temperature are the obligatory or facultative thermal windows. In mammals the eyes are always *obligatory* thermal windows, as are the mouth, heart region, and the rectal and vaginal openings, as well as the penis during urination or erection. These are areas where function permits no insulation, or where an opening in the body is connected with the body core. *Facultative*

thermal windows are much more difficult to judge because they may or may not be active, depending on the ambient conditions and the thermoregulatory needs of each animal. These are species specific and may also show individual variations.

Therefore, it is advisable to study many individuals over time before judging pathologic processes. When this is not possible, the investigator should make use of other individuals of the same species in the same environment, or if time permits, investigate the same individual on different occasions under similar conditions. This last approach yields the most accurate investigation technique for an individual. This is the technique used in equine preventive medicine or in racecourse training management, especially in Great Britain. Experienced trainers and veterinarians are able to identify potentially lame animals up to 2 weeks before the animal actually shows clinical signs.^{13,22,27}

General indicators of altered thermoregulation can be physiologic or pathologic, as follows:

1. Exposure to strong sun
2. High ambient temperatures with simultaneous high humidity and no water access
3. Physical activity
4. Stress (psychologic)
5. Pregnancy (see Monitoring Reproductive Events)
6. Abrasions (see Diagnosing Inflammation)
7. Inflammation

Elephants

As animals without a notable amount of hair on the body, elephants display relatively even heat radiation over their entire body surface when in a thermoneutral zone. Only the ears show less heat radiation than the body, whereas the eyes, mouth, and anus are thermal windows (Figure 3-1). Any other source of heat should

be investigated. A thermogram of an elephant feeding on branches may show the “hot” mouth, the hot distal trunk, and the warm tips of the front feet. Thermograms of an African (*Loxondonta africana*) and an Asian (*Elephas maximus*) elephant may show heat radiation with specific reference to their ears. In both animals the larger blood vessels are localized.

Intense sunshine creates high temperature readings on the body and outer surface of the ears, especially in African elephants. The underside of the large ears usually remains cool. Ear flapping results in increased convection and saves the ears from collecting further heat. More than 30% of excess heat may be radiated off the ears in African elephants.¹⁶ In a group of Asian elephants in a newly built indoor enclosure, the keepers noted that the elephants did not display normal activity patterns but seemed somewhat lethargic. IR thermography revealed an altered thermoregulation, with ears that were the same high temperature as the body. This was noted in all members of the group. The ambient high humidity of 95% was reduced, and the animals were given more frequent access to cool water. Overheating poses a great stress and health risk to captive elephants, especially Asian elephants, and may even cause death during immobilization, if the thermoregulatory influence of a new enclosure is not evaluated; in this case the health of the animals improved.⁹ Uhlemann^{25,26} provides similar examples of recent investigations into thermoregulatory behavior of zoo animals involving insight into environmental heat stress caused by enclosure design.

Elephants may also display increased radiation from parts or whole ears caused by psychologic stress. When this occurs, at least some animals in the herd display normal ear radiation and serve as comparisons.⁹

Rhinoceroses

As another species with lack of a significant hair coat, except for the Sumatran rhinoceros (*Dicerorhinus sumatrensis*), rhinoceroses kept at most zoos belong to one of three species: black (*Diceros bicornis*), white (*Ceratotherium simum*), or greater one-horned rhinoceroses (*Rhinoceros unicornis*). As with elephants, rhinoceroses display an even radiation over their body surface and legs, with only their ears and horns showing less radiation under normal conditions (Figure 3-2). Juvenile or newborn rhinos display a higher radiation than adults. This contrasts with newborn horses, because foals have long hair, which insulates the small body. In the greater one-horned rhino the thicker skin plates are visible as areas with less radiation.



Fig 3-1 Normal thermogram of an elephant, with the ear showing less heat radiation than the body.

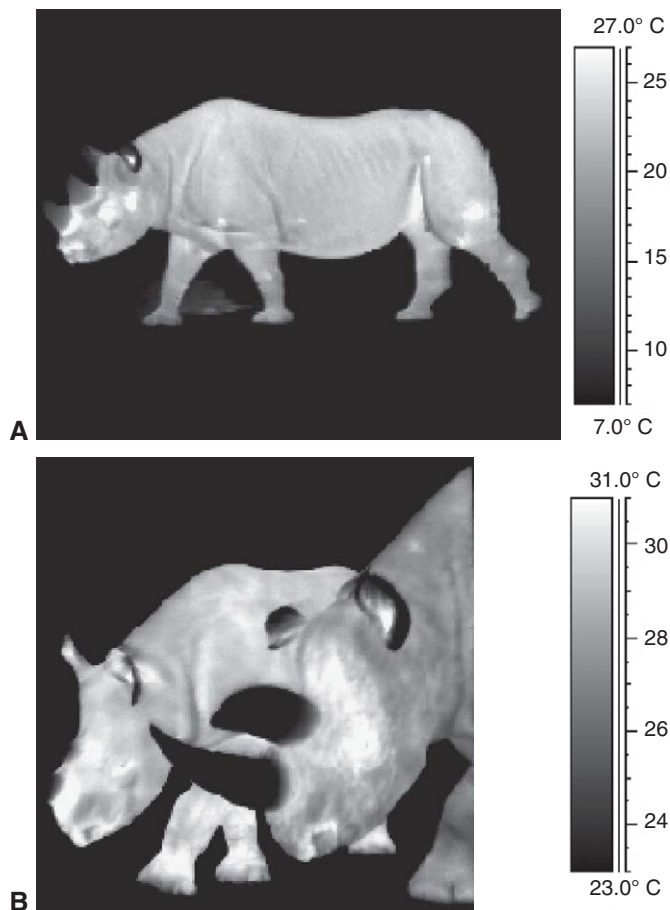


Fig 3-2 **A**, Normal thermogram of black rhinoceros. **B**, Ears and horns appear cooler than bodies of black rhinos.

Intense physical activity may create various forms of heat radiation in the animal under study. Activities such as mating in black rhinoceroses may cause the male partner to radiate intense heat over his whole body, whereas the female stays “cool” (Figure 3-3). Running creates heat in the shoulder and hip muscles, as well as in the legs. In animals with heavy heads, the head may also show increased radiation during running. In rhinos, only the head itself increases in radiation, whereas in deer the neck also shows increased radiation. Under normal circumstances the abdomen remains at the general body temperature and does not show increased radiation with a running activity, or only after prolonged activity. This is important for pregnancy diagnosis.⁹

Lions

The mane in adult male lions poses a specific challenge for thermography because it serves as an insulator. Only about 50% of the male lion’s body surface is available for temperature regulation because of the mane. Therefore a male lion could experience a heat

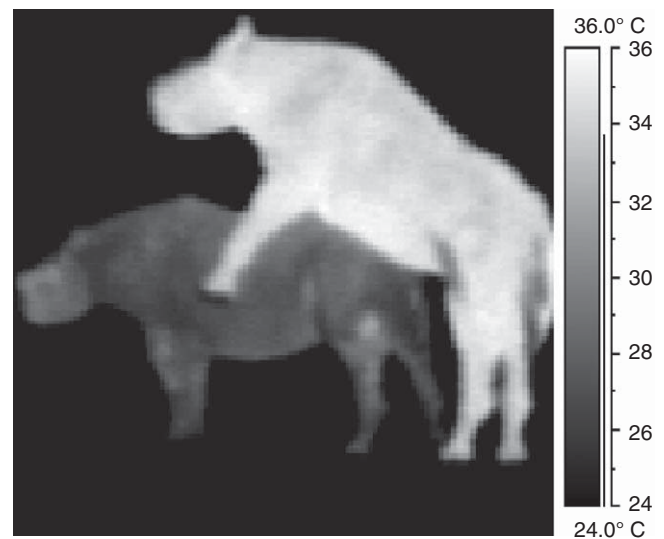


Fig 3-3 During mating, male rhinoceroses may be much warmer than female rhinos. (See Color Plate 3-3.)

stroke if hunting under intense sunlight.⁹ This was verified and placed into an evolutionary context in an investigation on wild lions in East Africa,³⁰ as well as in historical perspective.¹⁴

Anesthesia. An interesting feature in lion thermoregulation is observed during anesthesia (Figure 3-4). When the animal is under full anesthesia using a combination of medetomidine and ketamine, the nose is cold compared with the body. A few minutes after the antidote atipamezole is administered, the nose starts to warm up. When the nose is much warmer than the body, the lion may raise its head and soon arise. Therefore the nose temperature serves as a good indicator of immobilization status, and thermography may be used to monitor anesthesia.

Giraffes

Again, species-specific skin coloring has an influence on thermographic investigations in giraffes. The sun heats up the darker skin parts more intensely than the lighter parts, but even during the night the giraffe may display this same skin radiation pattern, even though no sun was present for hours, and a new equilibrium should have been reached (Figure 3-5). Therefore I investigated this phenomenon further. In a Rothschild giraffe (*Giraffa camelopardalis rothschildi*), a subspecies with three different hair colorings, the black-haired areas showed a less dense hair covering and a thinner epidermis than the white areas. In the superficial blood vessels, we found no difference with reference to the skin color.¹⁰ An earlier investigation suggests a skin color-related distribution of the slightly deeper

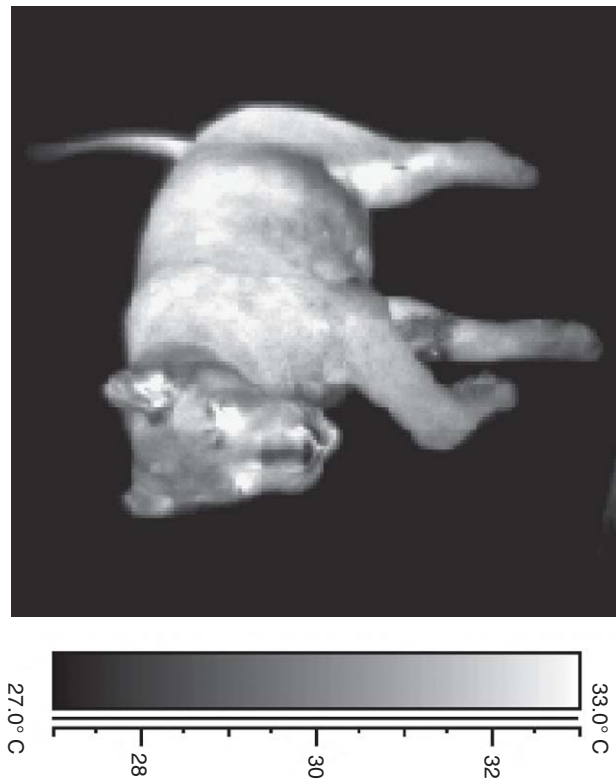


Fig 3-4 Thermogram of a female lion during anesthesia. Males show significant insulation in the mane region and little insulation on the remaining body surface.

and larger blood vessels.²¹ This thermographic, histologic study allows definition of the dark patches in giraffes as general, "predefined facultative thermal windows," which may be turned on or off, depending on local thermoregulatory needs.¹⁰

Zebras

Zebras show a different thermoregulation than giraffes in regard to the influence of the skin color. Zebras are similar to giraffes regarding influence of the sun. Under the bright sun a zebra shows higher radiation over the black stripes versus the white stripes, as well as a more intermediate radiation over brown stripes. Thus the hot black stripes show up in gray-coded thermograms as light areas, and the cooler white stripes appear as darker areas. In a Chapman zebra (*Equus quagga chapmani*) a maximum temperature of 71.9°C was measured on a sunny day of 22.8°C ambient temperature and 50% relative humidity. The average difference between black and white stripes was more than 20°C under these conditions. More surprising were the findings in the various zebra species at different zoos during night investigations. With no influence of the sun, the white stripes emitted more radiation than the black stripes¹ (Figure 3-6). This phe-

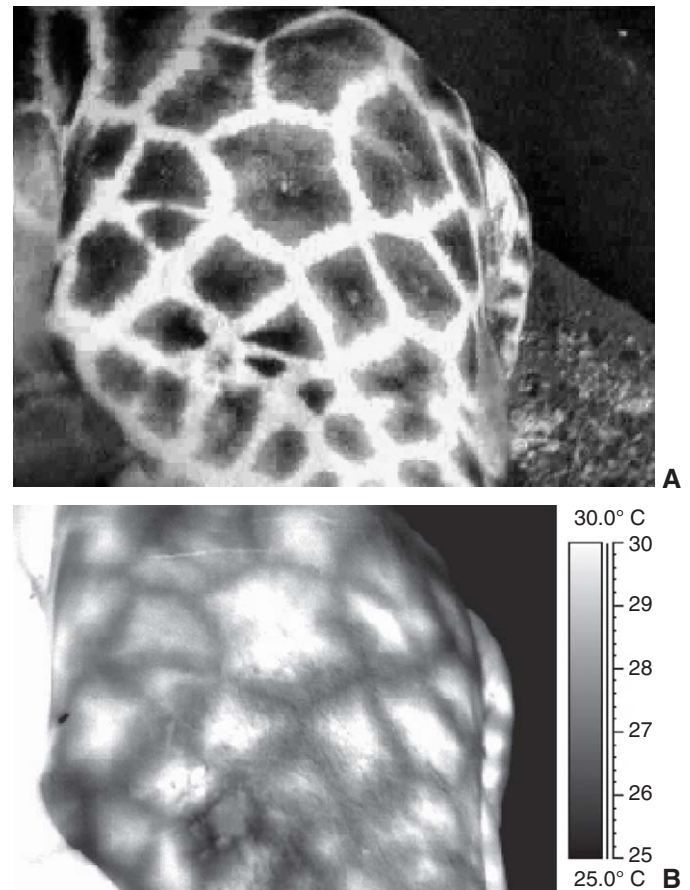


Fig 3-5 **A**, Thermoregulation in a giraffe during the day. **B**, Same individual during anesthesia. The dark spots radiated more heat than the white lines.

nomenon is explained by Kingdom,¹¹ who found insulating fat layers under the black stripes in plains zebras (*Equus burchelli*).

Other Animals

During more general studies using IR thermography, housing conditions were investigated in regard to their influence on the behavior and well-being of zoo and wild animals. In one investigation, Uhlemann²⁶ found significant heat stress for Mishmi takin (*Budorcas taxicolor taxicolor*), a large ruminant, from the rock surface of its enclosure. Temperatures greater than 60°C on the rocks resulted in the animals crowding into a small part of the enclosure covered with wood chips. Because takins are normally found in cool mountain environments, this indicated suboptimal management for this species and could pose medical problems from overheating.²⁶

In a study of wild greater mouse-eared bats (*Myotis myotis*), Sandel et al.¹⁷ discovered a temperature-related movement pattern. As ambient temperature

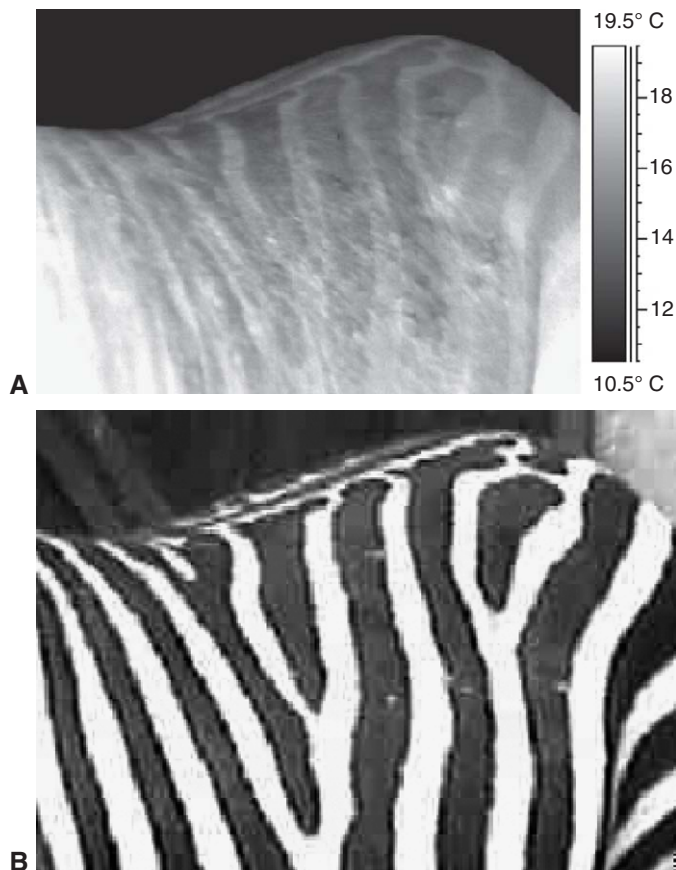


Fig 3-6 **A**, Thermoregulation in a zebra during the day. **B**, Same individual at night, when the zebra radiated more heat from white than from black stripes.

increased in the summer, the bats moved from positions under the tiles onto the wooden roof constructions, and with further temperature increase, onto the thick stone walls of the church roof. This choice of roosting places on a temperature gradient seemed essential for the temperature regulation in bats and is currently being investigated for zoo animals.

These examples illustrate that IR thermography may be a valuable tool in monitoring animals with regard to their health status and their surroundings in captivity, as well as in the wild. This technique helps veterinarians evaluate the habitats and welfare of the species in their care. The example of the herd of elephants revealed a true veterinary concern for monitoring enclosure design. For this more general application, however, one need not be a veterinarian to make sensible diagnoses. In the field of thermoregulation, a curator or technical personnel may be trained, or even an outside thermographer hired, to do an enclosure evaluation with the animals present. However, it is always advisable to have this done in cooperation with the zoo veterinarian.

Monitoring Reproductive Events

Unfortunately, not every zoo or park has the means to monitor reproductive events in elephants and rhinoceroses on a routine basis, and therefore reproductive output in these endangered species is suboptimal in some institutions. Researchers and zoo veterinarians have designed noninvasive methods for monitoring cycling activities, including fecal hormone analyses¹⁸ and minimally invasive methods such as rectal ultrasonography.⁷ However, both these techniques are labor and time intensive. Under field conditions, such methods often are impractical, too expensive, or against the philosophy of noninterference practiced in many national parks. In these cases, IR thermography may yield instant results with a noninvasive method and brief time commitment. Because no method is perfect, however, thermography has disadvantages, as mentioned earlier, as well as in the following examples.

Cycling Activities in Elephants

In my experience with IR thermography, female elephants with increased heat radiation over the vaginal sheath were noted. If a bull was nearby, he always followed the females with this presentation. A similar intense radiation through the vaginal folds in black rhinoceroses during estrus was also observed when they lifted their tail to present themselves to the bull. Especially in elephants, this finding should be pursued with scientific investigation in regard to its use in estrus determination. Once the method is established, inexpensive instruments could be used by keepers or park managers to assist reproductive management. For rhinos, however, this is not practical, unless the animal is easily accessible and the tail can be lifted by hand to give full access to the vaginal folds. Figure 3-7 illustrates a female Asian elephant with increased radiation over her vaginal sheath.⁹

Pregnancy Diagnosis

During pregnancy the female animal shows increased metabolism that allows for the growth of the fetus. When energy of one form is converted into another form, some energy is always lost in the form of heat. Depending on the ambient temperature and relative humidity, this metabolic heat, as well as the heat of the placenta and the body heat of the growing fetus, is channeled to the outside of the mother's skin by conductance, especially when the fetus is pressed against the mother's body wall. This sets two constraints for



Fig 3-7 Estrus in an elephant. During presumed estrus in this Asian elephant, the vaginal sheath radiated more heat than the other body surface. The bull pursued this female intensely.



Fig 3-8 Late pregnancy in an Asian elephant. The abdomen bulged, and heat radiation increased both from that area and from the mammary glands. The heat radiation during late pregnancy was so great that the feet and trunk functioned as facultative thermal windows. In some individuals the feet may show swelling and increased radiation. (See Color Plate 3-8.)

the use of this method: (1) the fetus must be of a certain size so that enough heat is produced to become visible through conductance, and (2) the ambient temperature and relative humidity must be in a range that allows conductance of excess heat. Ambient temperature is optimal between 15° and 18° C.⁹

To date, the best species for pregnancy diagnosis using IR thermography are the black and white rhinoceroses. In rhinos we diagnosed a pregnancy at the end of the first trimester. These animals possess a tough skin that shows little expansion during pregnancy and therefore allows localized heat areas to be visualized with sharp edges. This is not the case in elephants or giraffes. Even so, their skin also contains keratin and tough fiber, their skin is more elastic, and their abdomen bulges greatly during pregnancy. This creates a more diffuse picture without sharp edges around the increased-radiation areas.⁸ Experience has shown that providing a sound diagnosis of pregnancy in a multiparous giraffe is difficult. For primiparous giraffes the method works well for experienced thermographers. However, as previously noted, the predefined facultative thermal windows in giraffes pose a problem. Figures 3-8 and 3-9 show late pregnancy in a multiparous Asian elephant and in a multiparous black rhinoceros, respectively.

Diagnosing Inflammation

Heat production in inflammatory processes is one of the cardinal symptoms of inflammation. IR thermography picks up this heat if the process is located close

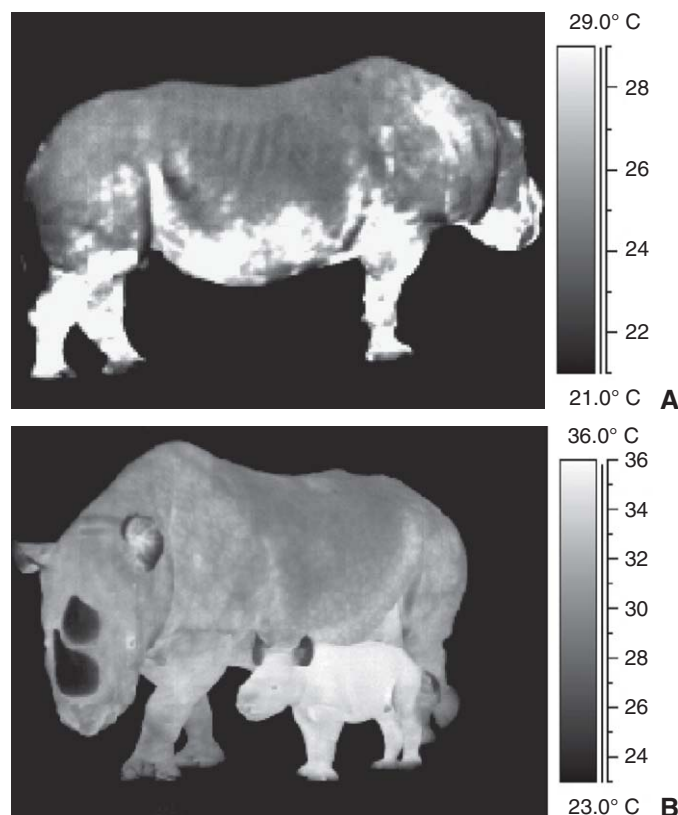


Fig 3-9 A, Reproductive evaluation in a black rhinoceros. Late pregnancy with increased heat radiation from the abdomen and legs. **B**, After the calf was born, the increased radiation disappeared in the mother but was shown by the newborn calf.

to the body surface. The diagnosis of an inflammatory process in a leg or ear is a good way to gain experience with this method. Figures 3-10 to 13-13 provide some examples.

Elephants

Elephants and giraffes have been major species for the use of IR thermography in inflammation diagnosis. Many problems are only visible using this new technology; even though some animals seem healthy under visual inspection, thermography may reveal a different picture. The following examples should encourage zoo veterinarians to employ this technology for their patients. Zoo animals are not domesticated, and thus they try to hide pain and illness when possible.

In the first case an Asian elephant displayed unsynchronized walking behavior, but no single leg or joint could be identified as the source of the abnormal gait (Figure 3-10). IR thermography revealed the problem to be the right foreleg, specifically the elbow. On the left side, no area of increased radiation could be found in the forelimb.

A major challenge for veterinarians and keepers remains the management of elephants in zoos because of the problems associated with their feet.^{2,5,12,20} Studies are ongoing to determine the prevalence of

foot conditions in wild elephants.²³ Even though elephant management has greatly improved, *pododermatitis* in elephants is still seen. New approaches to therapy have been presented.^{4,19}

In less severe cases of pododermatitis, only one nail shows increased radiation (Figure 3-11). If the therapy is effective, the inflammation will be reduced, and the nail will lose its increased radiation, as monitored by thermography. In more severe cases the whole foot emits increased radiation: the nails, interdigital glands, and the connecting tissues above the nail (Figure 3-12). From this stage the inflammation may quickly move to more proximal parts of the leg. The healing process usually takes years, so continuous,

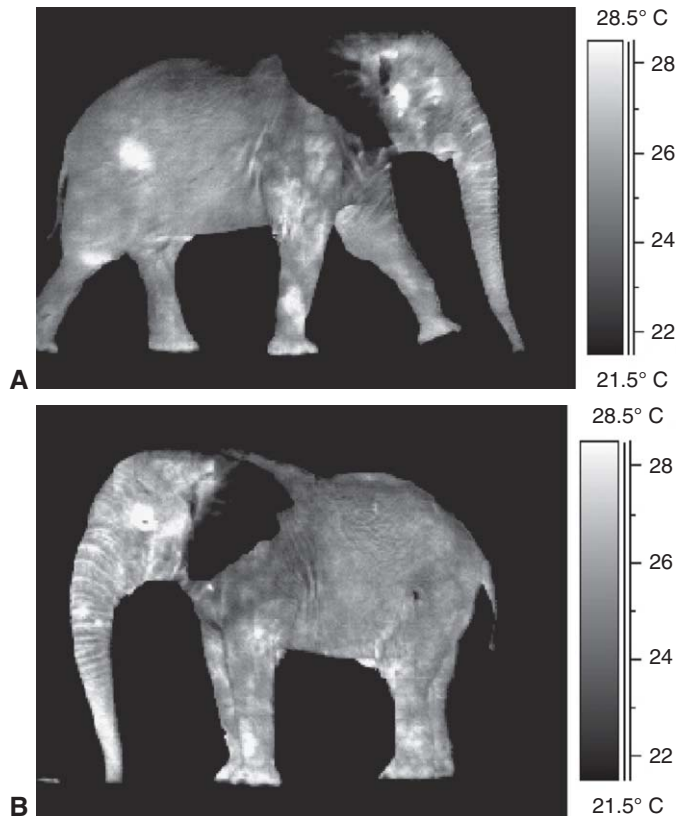


Fig 3-10 **A**, Inflammation in an Asian elephant. After an accident with the outside enclosure, this elephant went lame, but no location was found on normal diagnosis. Infrared thermography revealed that the location was on the right shoulder over the elbow joint. **B**, Left side showed no site of increased radiation.

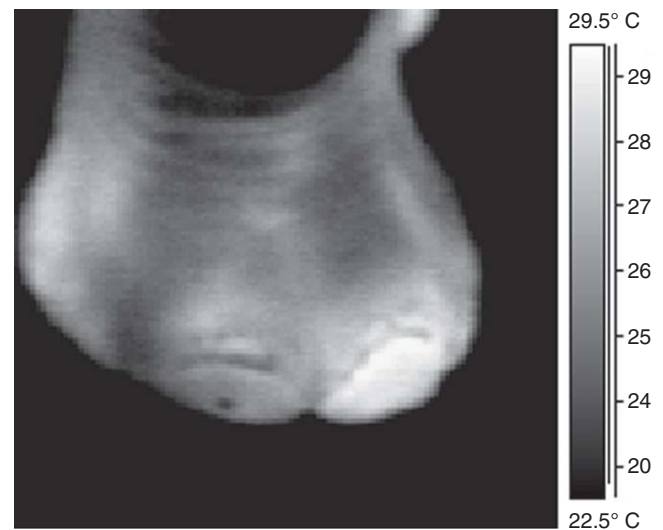


Fig 3-11 Chronic septic pododermatitis in an Asian elephant. The middle toenail in the front foot showed increased heat radiation. (See Color Plate 3-11.)

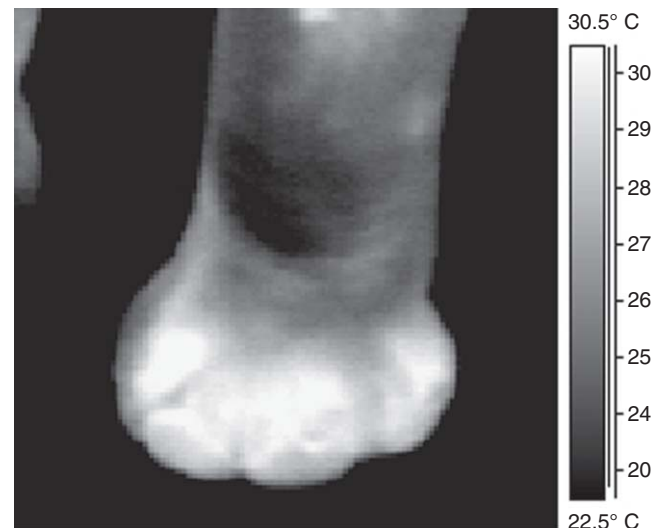


Fig 3-12 Chronic septic pododermatitis in an Asian elephant. The complete phalangeal region of the front foot displayed increased heat radiation.

regular treatment must be undertaken. In the patients, IR thermography helps to monitor the effects of the therapy.⁹

On rare occasions a completely different radiation pattern is found, as when an elephant's nails display greatly reduced radiation (Figure 3-13). This may indicate a severe necrosis of the nail, connective tissues, and even bone material, including osteomalacia and osteolysis. Shortly after finding this radiation pattern, one zoo decided to euthanize the elephant because it no longer used the foot and was therapy resistant. Pathologists found extensive necrosis up to the radius. All bones in the distal phalanges were completely lysed. Therefore, reduced radiation in an elephant's foot is a serious concern, and the foot should be radiographed immediately and the nail surgically explored. In another case the purulent exudates were found after thermography. (For the complete thermographic eval-



Fig 3-13 Nail abscesses in an Asian elephant. This elephant displayed severely reduced radiation in two nails of the right front foot and severe lameness. On opening of the nail base, large quantities of purulent material emerged. Euthanasia was performed after therapy resistance. Postmortem examination revealed lysis of all phalangeal bones in both nails.



Fig 3-14 This Asian elephant showed moderate lameness after a fight. Thermography revealed a bruise above the right carpal joint.

uation of Asian elephant leg and foot health, consult the website www.wildlife-thermography.com.)

In another example, an Asian elephant group had to fight for a new hierarchy. One female was limping with her right foreleg, but the severity of the injury was unclear. Thermography showed increased heat radiation just above the carpal joint, apparently the result of bruising from the metal stable dividers (Figure 3-14). The animal had been observed sticking her feet through the bars to strike at another animal. Subsequently, the animal was successfully treated locally with antiinflammatory ointments.

Hippopotami

In hippopotami the normal diagnostic technique is altered. The animal should be in the water for at least 1 hour before thermo-diagnosis is performed. To obtain an optimal reading, the thermographer should measure the body surface temperature immediately after the animal emerges from the water. Hippopotami have a thick skin (~4-5 cm) that is penetrated by many fine blood vessels. Past experience has shown many individually placed, facultative thermal windows in this species, which may hide the true inflammatory site. This altered approach yielded the best results to overcome this problem.

As in other species, a thermogram of the healthy individual is the best reference for later diagnoses. The example given here shows a hippopotamus with severe lameness of unknown origin (Figure 3-15). Within 3 minutes of leaving the water and simultaneous thermal imaging, the right carpal joint showed a 3°C higher temperature than the left side. The animal was



Fig 3-15 Hippopotamus with severe lameness. Infrared-thermography found that the location of the injured area was the right carpal joint. Within 2 minutes out of the pool, the animal displayed an increase in radiation of more than 3°C over the medial side of the right carpal joint. A hairline fracture was presumed after healing and reduction of the heat radiation took almost 2 years.

to be medicated as well as “stall rested” in the pool until the temperature difference was 1° C or less; this took 2 years. A hairline fracture or chip fracture in the carpal joint was suspected. Therefore, continuous surveillance of the animal allowed for informed decisions and constant evaluation of the efficacy of therapy.

Rhinoceroses

Rhinoceroses are usually not in direct contact with their keepers, so diagnosing lameness may be difficult. A black rhinoceros was presented with lameness in the left foreleg. Using the IR camera, however, the area of concern was the tarsus and stifle of the right hind leg (Figure 3-16). The lameness and elevated radiation observed in the front leg were compensatory. No superficial skin changes were visible, and the reason for this inflammation remained unclear. Because the animal was old, it was decided not to anesthetize it for further investigations, but rather try conservative treatment with local antiinflammatory ointments, and the lameness resolved.

Giraffes

In giraffes, hoof alterations are a common problem of animal management. Giraffes are challenging candidates for anesthesia, so they less frequently receive close examinations for leg or hoof problems than other hoofed mammals. With IR thermography, a surveillance program for giraffes may be installed with no risk and may assist in decision making for further procedures. Zoo veterinarians may start such programs by collecting routine thermograms of each individual in the herd. When alterations are visible, the surveillance may be intensified or other diagnostic tools utilized. Figure 3-17 illustrates leg problems in a giraffe observed at Frankfurt Zoo in which IR thermography assisted the evaluation of and surveillance for an inflammatory process.

In captivity, giraffes often develop hoof overgrowth, or alterations of the relative positions of the hoofs to each other. This results from inadequate hoof wear, which can be secondary to enclosure design (e.g., soft material such as loose sand), too little physical activity on hard surfaces, genetic predisposition, or nutritional factors. A recurrent, progressive, therapy-resistant lameness is often the reason for euthanasia of zoo giraffes. Postmortem, animals are diagnosed with arthritis and arthrosis.⁹

As a first measure, a surveillance program was installed at Frankfurt Zoo using thermography. The activity patterns of each member of a giraffe herd

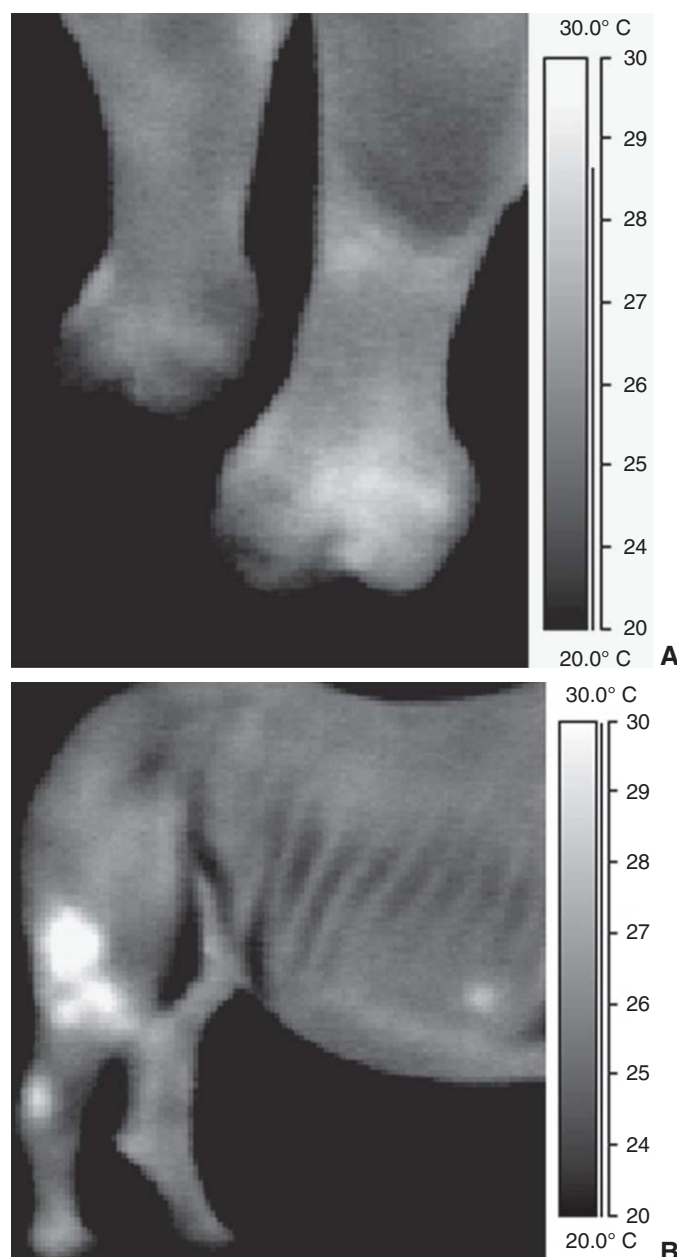


Fig 3-16 Lameness in a black rhinoceros. This animal was presented with lameness of the left front foot. **A**, The foot showed only a slight increase in heat radiation. **B**, The right knee/thigh area, however, showed an intense increase in radiation. A severe thigh bruise was the presumed cause of compensatory lameness in the front foot. (See Color Plate 3-16, B.)

(*Giraffa camelopardalis reticulata*) in relation to the enclosure were measured after it was discovered that several individuals showed increased heat radiation, with or without clinical lameness. A slight alteration in the enclosure had reduced the hoof overgrowth during the last few years but did not completely eliminate the problem. Figure 3-17 illustrates a giraffe breeding bull that became lame after pursuing two young bulls and tripping over a rock. On inspection the fetlock showed

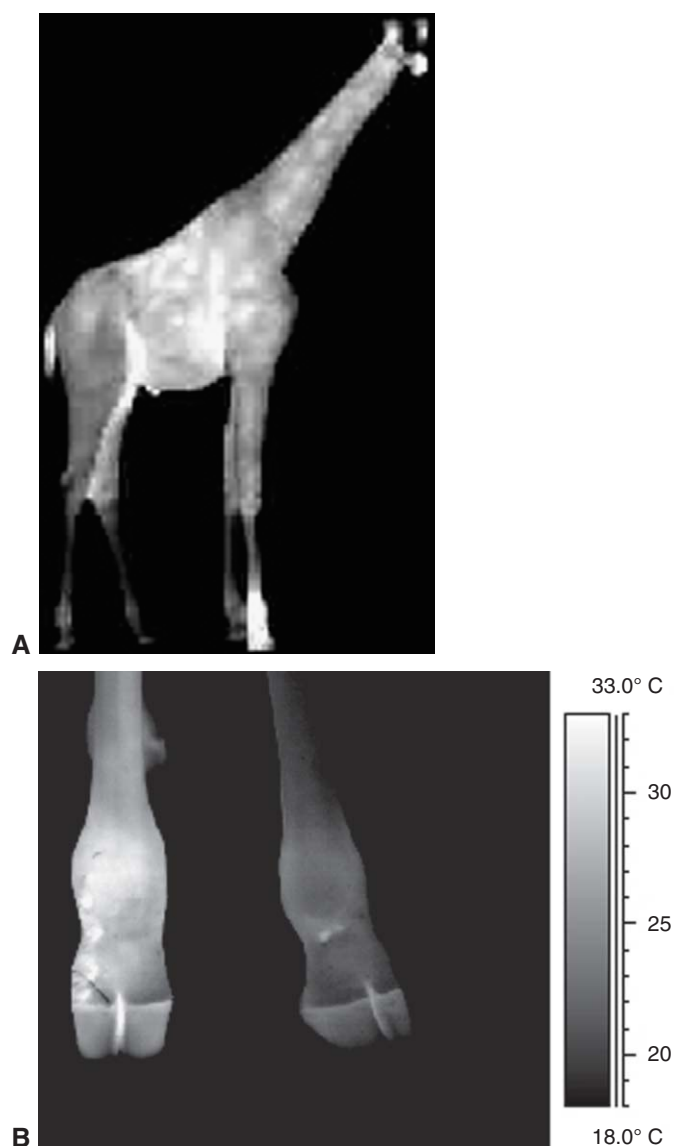


Fig 3-17 **A**, Giraffe bull after an injury to right fetlock. This bull had tripped over a stone in the enclosure and fell on his right side, causing abrasion and swelling on the fetlock. **B**, Only the fetlock and hoof area displayed increased heat radiation.

abrasions in three places; one surrounded a deep skin cut directly over the joint from which blood was seeping. Immediate immobilization of the animal seemed unwarranted because of the local facilities, so a simple external treatment was tried. The animal was sprayed with 20 mL of 3% hydrogen peroxide (H_2O_2) directly on the abrasions. The bull showed intermediate-degree lameness and swelling of the fetlock. IR thermography showed no further increase of heat radiation, and the swelling went down on day 3. In this case, thermography confirmed that other measures, including the risks of immobilization, were not warranted.

Other Animals

The method of IR thermography may be used for inflammatory investigations on other, smaller mammals as well. For example, observations have been made of a southern pudu (*Pudu pudu*) with a lameness over a distance of 5 m in its enclosure; a musk deer (*Moschus moschiferus*) with a second hairline fracture next to the primary fracture in the metatarsus observed in the x-ray film; a pygmy hippopotamus (*Hexaprotodon liberiensis*, formerly *Choeropsis liberiensis*) with tenosynovitis; Grevy's zebras (*Equus grevyi*) with various inflamed joints, hooves, or multiple inflammatory processes on the legs, even on wild animals under African conditions⁹; marine mammals with flipper problems; and a dolphin (*Tursiops truncatus*) with an abscess.

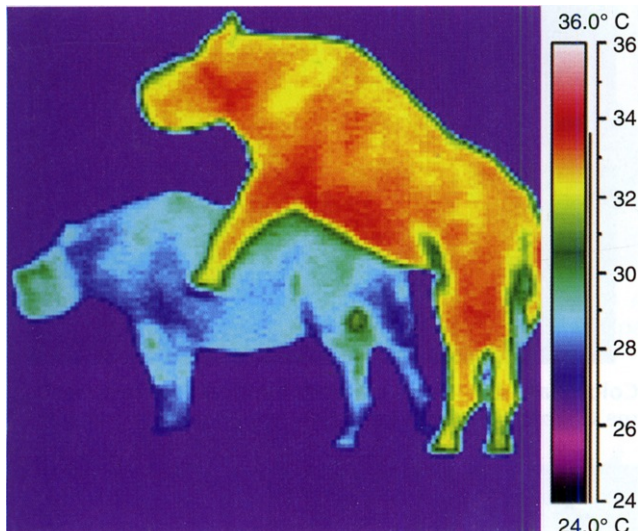
In birds, penguins have been the species of most intense use of thermography. In one exhibit a rock-hopper penguin (*Eudyptes crestatus*) was observed with severely increased radiation over the right foot. Clinical investigation showed a small, infected wound under the foot.

Descriptions of many other cases can be found elsewhere.⁹ The official website (www.wildlife-thermography.com) is being constantly updated to provide a place to share information among zoo veterinarians.

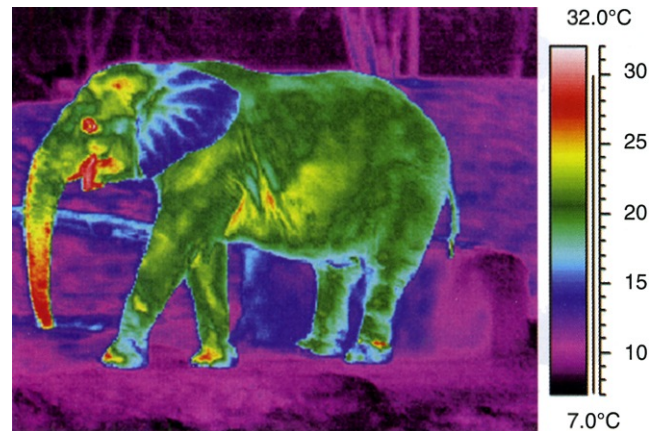
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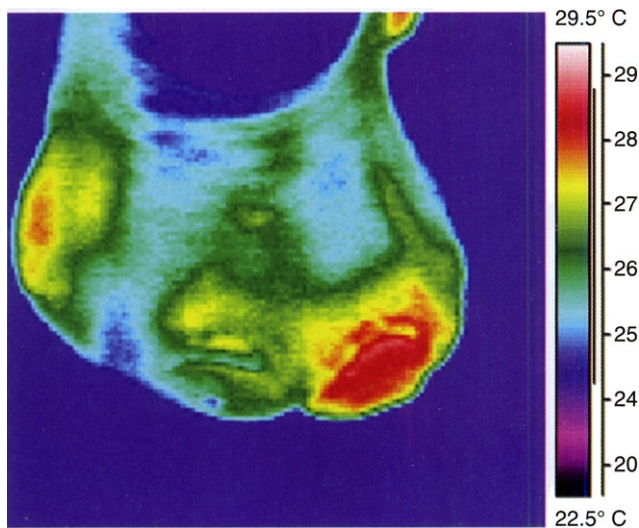
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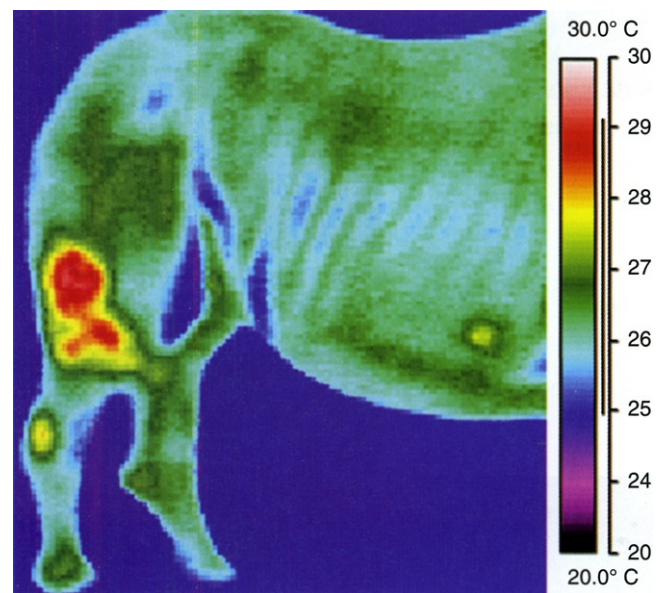
Color Plate 3-3 During mating, male rhinoceroses may be much warmer than female rhinos. (For text mention, see Chapter 3, p. 24.)



Color Plate 3-8 Late pregnancy in an Asian elephant. The abdomen bulged, and heat radiation increased both from that area and from the mammary glands. The heat radiation during late pregnancy was so great that the feet and trunk functioned as facultative thermal windows. In some individuals the feet may show swelling and increased radiation. (For text mention, see Chapter 3, p. 27.)



Color Plate 3-11 Chronic septic pododermatitis in an Asian elephant. The middle toenail in the front foot showed increased heat radiation. (For text mention, see Chapter 3, p. 28.)



Color Plate 3-16, B Lameness in a black rhinoceros. The right knee/thigh area showed an intense increase in radiation. A severe thigh bruise was the presumed cause of compensatory lameness in the front foot. (For text mention, see Chapter 3, p. 30.)

CHAPTER 4

Behavioral Clues for Detection of Illness in Wild Animals: Models in Camelids and Elephants

MURRAY E. FOWLER

As often noted, wild animals may be in an advanced state of disease before clinical signs are evident. Wild animals are not immune to pain or discomfort, but they do attempt to mask overt signs that would reveal their physical condition. Wild animal veterinarians should make every effort to diagnose disease at its earliest stages. Therapy may be useless unless it is initiated early in the course of a disease.

This chapter focuses on camelids and elephants in discussing normal and altered behavior in relation to the health and well-being of the animal.^{4,5} The influence of behavior on the health of animals is not a new concept, but it has become an important facet of veterinary medical education only during the last two decades. Several disciplines use behavior as a basis for study, including psychology, ethology, sociobiology, and animal behavior. Numerous contemporary authors discuss basic animal behavior and clinically abnormal behavior.^{1,10-13,16,25}

Altered behavior is a key to detecting incipient illness. Each species or animal group has a repertoire of actions that astute observers are capable of evaluating and classifying. For purposes of this discussion, behavior is defined as all aspects of an animal's total activity, especially those that may be externally observed. Behavior may be controlled by genetics, in which case the action is innate, but may also be learned or modified by individual experience.

Zoo and wildlife veterinarians may deal with hundreds of species of animals, each with their own behavioral characteristics. How then can they know all the subtleties of behavior that would allow them to detect early clues of altered behavior? In short, they cannot, but there are basic behavioral patterns that are

shared by most mammals. Birds have their own patterns, as do reptiles and amphibians. Veterinary students become well versed in physical examination and laboratory detection of illness, but many receive little training or experience in simply observing normal behavior in a natural setting for domestic animals, let alone wild species. So how does one acquire the skills that will enable a person to detect the early stages of illness? One may read about behavior, but it takes time just looking at the species in a collection to learn enough to determine even minor variations from "normal" behavior. Another method is to listen to experienced keepers and trainers, but personal observation remains a key element.

Acquiring observational skills is important for the following reasons:

1. To be able to detect incipient illness.
2. To detect stress in the lives of wild animals.
3. To assist in the welfare and well-being of wild animals.
4. To be able to advise wisely in the construction of new enclosures.
5. To help train keepers to identify altered behavior.

A veterinarian must first understand normal behavior to be able to detect abnormal behavior. Behaviors to be included are methods of offense and defense; communication (vocalization, body language, facial expression), social behavior, interaction with other animals, hierarchic status, locomotion, food intake, defecation/urination, scent marking, recumbency, getting up and down, reproductive behavior (courting, copulation), and stress.^{8,14,16,23,27}

CAMELIDS

Normal Behavior

Offense and Defense

Offense and defense weapons used by camelids include kicking, charging, chest butting, biting, and spewing stomach contents (spitting) onto other camelids or people (Figures 4-1 and 4-2). Veterinarians and animal handlers must be aware of any abnormal



Fig 4-1 Male camelids fighting, chest butting. (See Color Plate 4-1.)



Fig 4-2 Male camelids fighting. (See Color Plate 4-2.)

behavior that may develop in hand-raised camelid neonates. Camelid neonates (*cria* in South American camelids [SACs], Spanish term for “baby animal,” and *calf* in Old World camelids [OWCs]) that are bottle-fed and kept without social intercourse with other camelids may become imprinted on people. The resulting abnormal behavioral characteristics are more critical in male camelids but may also occur in females. When the hand-raised male reaches sexual maturity, he may begin to treat humans as he would another male camelid. He will charge and chest-butt a person, who will likely be knocked down and then bitten. When male camelids fight other male camelids, they may bite each other on the legs, neck, or more seriously the scrotum, castrating the victim.

This situation occurs more often in privately owned camelids, but if a *cria* is placed in a zoo petting area with only human companionship, problems may develop at maturity. I was attacked by a hand-reared male dromedary camel, which charged open-mouthed. Fortunately, a lead rope warded off the attack until escape was possible.

Spitting behavior is, unfortunately, one of the few facts that the general public knows about SACs. They are capable of projecting the foul-smelling stomach contents a distance of 1 to 2 m (6½ ft). SACs spew forward, but OWCs may also “spit” out of the side of their mouths. In reality, llamas and alpacas are generally placid around people, and spitting at people is rare. If milder threat displays are disregarded, however, spitting is the ultimate response in social intercourse between SACs. The material spewed out of the mouth may be saliva or feed material, if in the mouth at the time. It is interesting to watch an annoyed *cria* spew out a vapor of saliva. The reflex response exists, but the first compartment of the stomach is not yet functioning, so there is no content other than saliva.

The behavioral sequence of spitting begins with the ears laid back against the neck, accompanied by a gulping or gurgling sound from the throat region. A bolus of food is then regurgitated from the first compartment of the stomach.

It has been my experience that alpacas are more prone to spitting than are llamas, but individual llamas may also develop a dislike for a particular person. Veterinarians often bear the brunt of such disfavor.

SACs usually “cow kick,” reaching forward and outward. OWCs may reach forward and strike with the foreleg and may kick in any direction with the hind leg. Camelids have long legs and may scratch an ear with a rear foot. Alpacas tend to be more prone to kicking than llamas.

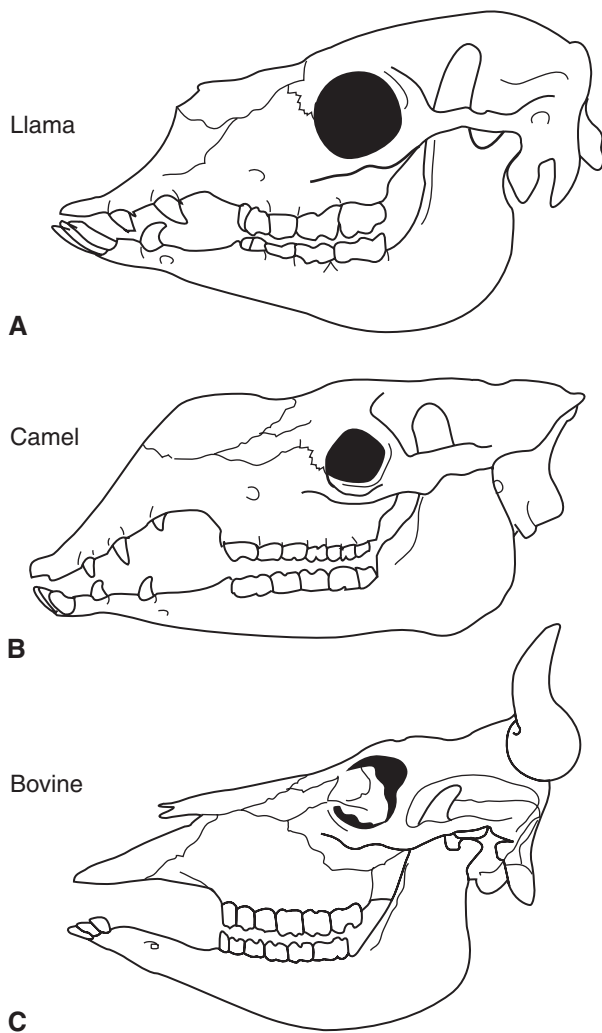


Fig 4-3 Male camelid skulls: **A**, llama; **B**, camel; **C**, bovine.

Male camelids have formidable canine teeth and are capable of inflicting serious or fatal injury (Figure 4-3). OWCs have been known to grasp a child by the head and shake it, often breaking the neck and crushing the skull.

Communication

As in human society, an effective means of communication is vital for the survival of any population of wild or domestic animals. SACs communicate with each other, humans, and other animals by vocalization and body language and by scent (see Scent Behavior).

Vocalization. Although SACs are not highly vocal, they do have a repertoire of sounds. Alpacas are generally more vocal than llamas. The most common sound has been described as humming (bleating). The pitch and tone of the humming are significant in SAC communication. Franklin⁷ describes the “contact

hum” as an auditory contact between herd members and especially between a mother and her cria. “Status humming” is a deeper tone that communicates contentedness, tension, discomfort, pain, or relief. The “interrogative hum” is higher pitched and has an inflection at the end. Other variations in intonation are described as a “separation hum” or a “distress hum.”

Llamas emit a *snort* characterized by a short burst of air through the mouth with loose lips. The snort indicates mild aggression. A clicking sound may be made with the tongue, which also indicates mild aggression. A grumbling threat is emitted when a feeding animal is approached too closely by another, or when an aggressor is about to regurgitate onto an offender.

Screaming indicates extreme fright. Some llamas and alpacas scream continuously when restrained for diagnostic or therapeutic procedures. *Screeching* is a loud squealing sound, usually made by males chasing one another during a territorial dispute or a fight.

The SAC *alarm call* is emitted when a male or female perceives danger to be near. The approach of strange dogs or other predators may trigger an alarm call. The alarm call is a high-pitched series of sounds variously described as “whistling” or “neighing” and by some as the “braying of a hoarse donkey.” When the alarm call is sounded, other SACs within hearing become alerted and turn toward the source of the sound.

Male llamas, alpacas, and guanacos emit a rhythmic expiratory grunting sound called *orgling* while chasing a female or copulating. Vicuñas may or may not orgle. The word “orgling” is not in a dictionary but is in common usage by people involved with camelids. An owner coined the term, which is a phonetic approximation of the sound made.

Body Language. Body language, including ear and tail position, is a sure indicator of the mental state of a SAC. Various degrees of aggression are communicated between herd mates by ear, head, and tail positions, usually displayed in concert (Figure 4-4). The ears of a contented, nonaroused SAC are in a vertical position and turned forward. In the alert animal the ears are cocked forward; relaxed SACs may allow the ears to lie horizontal to the rear (Figure 4-5). This is a normal position and should not be considered aggression because other signs of aggression are absent. In some individuals the ears may appear to spread sideways from the top of the head. This ear position may be used when listening to activity behind them or just for relaxation. Asymmetric ear positions may also be seen. Ear and tail position may be in a continual state of flux, especially when animals are fed and if feeding stations lack adequate space for all herd members.

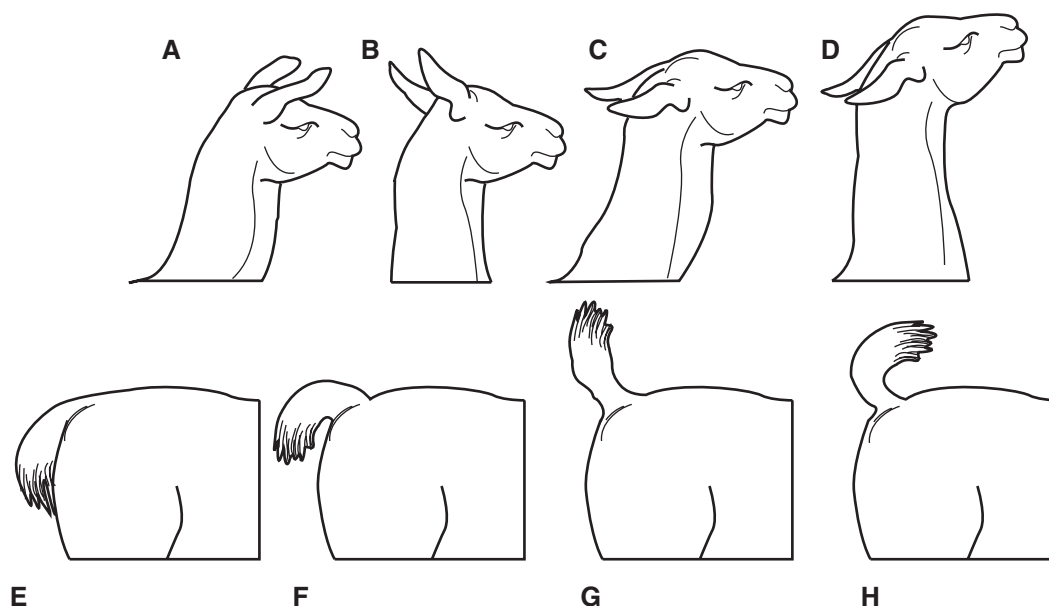


Fig 4-4 Camelid ear and tail positions: **A**, normal alert; **B**, slight aggressive; **C**, aggressive; **D**, extreme aggression (threat); **E**, normal relaxed; **F**, alert tail stance; **G**, alarm tail stance; **H**, aggressive tail stance, also submissive tail stance.



Fig 4-5 Ear position: alert in back, mild aggression in front. (See Color Plate 4-5.)

Mild to moderate aggression is signaled by the head held horizontal, with the ears positioned above the horizontal. As aggression increases, the ears are below the horizontal and may be flattened against the neck. Intense aggression is exhibited by the nose being pointed in the air and the ears flattened against the neck.

Camel ears are much shorter but may still be observed in the same positions as for SACs, signaling similar emotional states.

Tail position also communicates social information. In the nonaroused SAC, the tail lies flat against the body. Mild aggression or alertness is indicated by the tail being slightly elevated, but below horizontal.

As the degree of agitation escalates, the tail may be carried horizontal, curled above horizontal, or vertical. Basically, the higher the tail, the higher is the level of aggression. The tail may also be seen to wave from side to side, especially in males that are slightly agitated. These aggressive behaviors are employed by social animals to minimize outright fighting.

Submissiveness in the llama, guanaco, and alpaca is indicated by curving the tail forward over the back, with the head and neck held low, the ears in a normal to above-horizontal position, and the front limbs slightly bent (Figure 4-6). This behavior is frequently seen in SACs that become imprinted on humans. The



Fig 4-6 Submissive crouch. (See Color Plate 4-6.)



Fig 4-7 Four South American camelids: *top left, vicuña; top right, guanaco; bottom left, alpaca; bottom right, llama.* (See Color Plate 4-7.)

submissive crouch of a vicuña is with the tail curved forward, but with the head curved back over the body.

Llamas generally move at normal gaits with the head held vertically or slightly forward. Alpaca normal neck position is approximately 70 degrees above horizontal. When either of these species rushes or charges at dogs, coyotes, other SACs, or humans, it does so with the neck held almost horizontal. This position may be used for balance, because it is also the head and neck position used when running downhill.

Social Behavior

All four species of SACs are social animals (Figure 4-7). Alpacas are generally more flock or herd oriented than llamas. Alpacas are also shyer, more easily frightened, and less curious than llamas. Wild SACs (guanacos and vicuñas) live in social groups.

Vicuñas have separate family feeding and sleeping territories defended by a single adult male, with a few breeding females and their young offspring. The territories are delineated by strategically located dung piles and perhaps other scent-marking stations. Pathways between feeding and sleeping territories may be shared with other family groups. Juvenile and subadult males live in bachelor herds.

Guanacos may be sedentary or migratory. They also have feeding territories, live in family groups during the breeding season, but disperse or seasonally migrate into larger social groups during the non-breeding season.

Domestication of llamas and alpacas has modified intense territorial behavior, but most of the communication forms have been retained. Separation of an individual from a SAC herd should raise a “red flag,” warranting close inspection and examination.

Interaction with Other Animals and People

Llamas and alpacas are curious about the presence of any strange animal in their environment. Their curiosity may lead to trouble if an animal investigates the presence of a venomous snake, and nose bites are common.

Dogs are tolerated if they are familiar farm dogs. Large dogs, such as maremmas, Great Pyrenees, and Anatoli sheepdogs, are used to guard llamas and alpacas from marauding coyotes and other dogs. Such guard dogs live with the animals and may even be seen to herd a SAC away from danger. Strange dogs always elicit an alarm stance or even an alarm call to bring the herd to attention. If the dog enters the enclosure, a single animal may attack it, or the entire herd may face the dog and rush it in concert.

Dogs are occasionally used to herd alpacas, but camelids generally do not tolerate this well. A dog that approaches from the rear is likely to be soundly thumped by a flying foot unless it is experienced in avoiding the rapid-fire kick of either llama or alpaca. If the dog approaches from the front, it likely will be charged. SAC aversion to dogs carries over from the attitude toward other would-be predators that could

threaten juvenile camelids. North American predators include packs of dogs (*Canis familiaris*), coyotes (*Canis latrans*), and mountain lions (*Felis concolor*).

Hierarchic Status

A group of SACs, whether all males, all females, or of mixed gender, quickly establishes a hierarchy ("pecking order"). Hierarchic status may be determined by seniority in the herd, age, or gender and relatedness. Once established, the rules are obeyed, or action is taken by the dominant over a subordinate individual. The action may be only a threat or may be carried to a conclusion by spewing stomach contents at the offender. However, adult males may engage in vigorous combat.

Defecation and Urination

South American camelids normally urinate and defecate at communal dung piles (Figure 4-8). The ritual begins when first arising in the morning at daybreak. This may be the only time that urine samples and fresh fecal samples may be collected. The dung pile is a social gathering site. Camels defecate indiscriminately. Camel fecal pellets are so dry that they may be used for fuel immediately on discharge.

Male and female camelids partially squat and project urine rearward, clear of the hind limbs. Changes in frequency, position, and duration of urination and defecation are important indicators of incipient illness.

Grooming and Water Behaviors

Rolling or dust bathing is one form of grooming in SACs (Figure 4-9). This behavior is so innate that pack llamas with full packs have been seen to lie down and attempt to roll. It may be similar to scratching one's back. In addition to dusting, grooming involves other behaviors, such as scratching with a hind foot on the



Fig 4-8 Llama defecating. (See Color Plate 4-8.)



Fig 4-9 Llama dusting. (See Color Plate 4-9.)

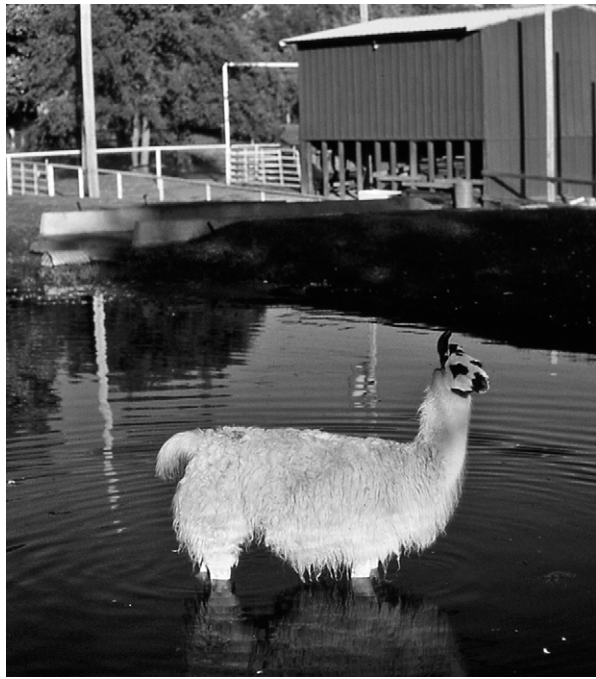


Fig 4-10 Llama standing in a pond to cool off. (See Color Plate 4-10.)

bottom of the abdomen, front limb, or head and neck; rubbing against fence posts, fencing, barns, or trees; and chewing at accessible points on the body or limbs. These behaviors do not necessarily signify a skin condition; it is often a simple itch.

Alpacas like to play in water. If water is provided in tubs, buckets, or tanks, they will joyously splash. Both llamas and alpacas seek out water during hot weather (Figure 4-10). If a pond or large water tank is available, they may stand in the water up to their abdomen. Both species are capable of swimming but do so only when forced; however, they will stand or lie down in shallow ponds or streams to cool themselves. Heavy fiber normally covers the legs of alpacas down to the fetlocks. In hot weather they may stand in water so long that the leg fiber becomes macerated and sheds, leaving a blocked-haircut appearance on the upper leg.

Locomotion

Locomotion is a form of behavior, and evaluation of locomotion patterns is important in assessing conditions of the musculoskeletal and nervous systems. Camelids have four natural gaits: the walk, pace, trot, and gallop. The long-legged llama is more prone to use the pace as a medium-speed gait, whereas the shorter-limbed alpaca tends to gallop more easily.

Juvenile SACs often engage in play behavior; tussling with one another and, especially at twilight,

engaging in a fifth gait, a stiff-legged bouncing called *stotting* or *pronking*. Occasionally, young adults will also join in the activity, particularly females trying to attract the attention of males.⁷

If an animal is removed from a herd, or if a new grouping is formed, the stress level in the herd may be elevated until hierarchic status is reestablished. In most cases it is easy to spot the dominant individual in a herd, but subtle nuances are detected by carefully observing the animals eating at a manger. This is a good time to note the repertoire of ear and tail positions.

Food Intake

South American camelids spend many hours each day grazing or browsing and ruminating. Although llamas and alpacas are not ruminants in a taxonomic sense, they do ruminate (regurgitate a bolus of stomach contents, rechew the cud, and reswallow it). The progenitors of both camelids (suborder Tylopoda, “padded foot”) and ruminants (suborder Ruminantia) separated and followed different evolutionary pathways 40 to 50 million years ago, when both groups had simple stomachs. Parallel evolution brought them both to a foregut fermentation strategy to utilize highly fibrous forages.

The pattern of chewing is different from that in horses, cattle, sheep, goats, dogs, or cats, being a figure-eight configuration. The cheek teeth normally have sharp enamel points to assist in the shearing and grinding process. The cycle of ingestion of feed, regurgitation of a bolus by reverse esophageal peristalsis, rechewing, and reswallowing is a series of behaviors that merit notice by managers and veterinarians alike. Variations in the rhythm, rate, and characteristics provide valuable insight to digestive function.

Scent Behavior

South American camelids have unique, oval-shaped, hairless patches on both the inside and the outside surfaces below the hock on the rear limbs. Associated with the patches are scent glands, with ducts emptying on the surface. The function of these glands is probably the excretion of alarm pheromones, perceived as a “burnt popcorn” odor by humans. The glandular secretion solidifies on excretion into a leathery sheet on the surface of the skin that may be peeled off.

Interdigital glands (between the toes) are found on all four feet. The structure and specific function of these glands are unknown, but they are probably associated with individual and group identification.

Flehmen is a behavior exhibited primarily by males, occasionally by females, in which the animal raises the nose into the air, with the mouth slightly open, to facilitate pheromone detection by an odor detection organ in the roof of the mouth. *Pheromones* are substances secreted to the outside of the body and smelled by another animal of the same species, initiating a specific behavioral reaction.

Males housed with females may sniff at the dung pile after urination by the females, to pick up the scent of pheromones in the urine, which may indicate that a female is receptive for breeding. The male then may go about sniffing the perineal area of the females to identify precisely the receptive individual.

Recumbency

Sternal recumbency is the most common position for rest and relaxation for llamas and alpacas. In fact, this position is also considered the default position for



Fig 4-11 Sternal recumbency with front legs forward. (See Color Plate 4-11.)

them when faced with an unpleasant situation, such as toenail trimming or blood collection. When lying sternally, the front legs are usually folded beneath the chest, but SACs have the unique capability to lie with the forelimbs extended forward (Figure 4-11).

SACs have a pronounced callosity over the sternum, and they may remain recumbent sternally for hours to days without compromising the circulation of the limbs. OWCs have an osseous pedestal on the sternum, overlaid with a pronounced skin callosity. Lateral recumbency is also a normal camelid position, with the animal apparently sleeping or sunning itself through the thermal window. An evaluation of the forms of recumbency is important in disease diagnosis (Figure 4-12).

Reproductive Behavior

South American camelids have a unique reproductive physiology (induced ovulation) and an array of behaviors associated with the reproductive cycle; space does not permit a discussion of the details of reproductive behavior. However, assessment of both male and female behavior is important in evaluating fertility and infertility.⁶

Behavioral Changes Associated with Illness

Behavior altered from normal may signal the onset of discomfort or illness. Handlers and trainers are the key players in the early detection of illness. The rate, intensity, or character of a behavior may be altered (Tables 4-1 to 4-4). Many of the telltale indicators of illness are

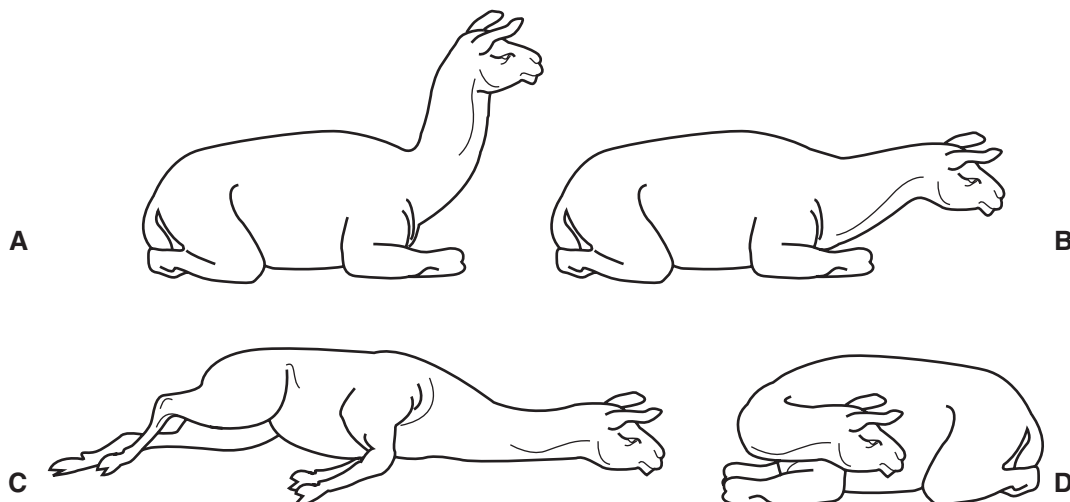


Fig 4-12 Recumbency in South American camelids: **A**, normal sternal; **B**, depression; **C**, normal lateral or depressed; **D**, opisthotonos, depression or serious illness.

Table 4-1**Exaggerated Normal Behaviors of South American Camelids**

Normal Behavior	Abnormal Behavior	Possible Causes
Lying sternally or laterally	Unable or unwilling to arise	Many general, neurologic, musculoskeletal, digestive, cardiovascular, or urogenital disorders
Humming	Excessive humming	Isolation from herd mates or crias; weaning time
Defecation at the dung pile	Prolonged squatting with little or no feces passed	Constipation, intestinal obstruction
	Multiple returns to the dung pile	Same as above, plus dystocia and uterine torsion
	Straining	Same as above
	Groaning	Colic in any form
Urination at the dung pile	Dribbling instead of steady stream	Partial obstruction of urethra, colic
	Frequent attempts to urinate	Urethritis, partial obstruction
	Abnormal stance	Nervous system and skeletal system disorders
Dust bathing (rolling)	Excessive rolling	Mild colic, displacement behavior, dermatitis
	Rolling accompanied by groaning	Colic, dystocia, intestinal obstruction, urolith
Grooming	Excessive scratching, chewing, and rubbing	Dermatologic conditions, external parasites
Bright, alert facial expression	Depression	Numerous nervous system disorders, septicemia, colic
Normal body stance	Stretching, cross-legged, leaning, pressing	Colicky pain, encephalopathy
Ear position	Asymmetric ear position	Facial paralysis, ear infection, nerve trauma
Tail position	Crooked tail, short or no tail	Tail trauma, congenital defect

Table 4-2**Diminished Behavioral Activities of Camelids**

Normal Behavior	Diminished Activity	Possible Causes
Vocalization	Lack of normal vocalization	Depression from multiple causes
Play behavior in herd animals	Failure to play with other herd mates	Meconium constipation, rickets, navel ill, septicemia
Intimate social contact	Separation from the herd	Normal for a female near parturition; depression; ostracism by herd mates; dominant male may drive a subdominant male from the herd.
Grooming	Failure to groom; matted fiber, hay, or straw in coat	Depression, failure to shear appropriately

an exaggeration of normal behavior. Other behavioral changes associated with illness may include the following:

1. Self-separation from the herd.
2. Normally docile individuals becoming aggressive.
3. Aggressive or dominant animals becoming submissive.

4. Changes in the frequency, posture, and productivity while defecating or urinating.
5. Prolonged recumbency.

Vocalization

The normal *humming* pattern for each individual is important background information. The character of

Table 4-3**Altered Anatomy and Behavior in Camelids**

Normal Behavior	Altered Anatomy and Behavior	Possible Cause
Normal gaits	Lameness Ataxia (incoordination) Angular limb deformity, mechanical lameness	Trauma, musculoskeletal disorders Spinal column trauma, meningeal worm Congenital defects of musculoskeletal system, nutritional deficiency
Normal vocalization	Groaning, grunting, tooth grinding	Colic
Normal hierarchic status	Continual fighting, aggression	Hormonal imbalance, pseudohermaphroditism
Dominance behavior	Becomes submissive	Loss of status in the herd, general illness, depression
Submissive behavior	Becomes aggressive	Juvenile male maturing, hormonal change
Bright, expressive eyes	Dullness, inattentive gaze	Depression, infectious diseases

Table 4-4**Camelid Recumbent Behavior as Indicator of Health or Illness**

Position	Possible Causes	Concern Level*
<i>Sternal</i> , bright, alert, able to hold head up, good appetite, able to stand	Healthy South American camelid (SAC), normal behavior	1
<i>Sternal</i> , head laid on the ground in front of the body	May be a normal resting or sleeping position, or may indicate slight depression (incipient sign of many illnesses if accompanied by other signs)	1-2
<i>Sternal</i> , anorectic, will not drink, will stand only if forced to do so	Slight to moderate depression	4
<i>Sternal</i> , head and neck held back over the thorax	Colic, brain disorders	5
<i>Lateral</i> , able to right self to sternal, bright and alert, able to stand	Healthy SAC; may be resting, sunning, or sleeping	1
<i>Lateral</i> , able to right self to sternal but unwilling or unable to stand	Slight depression (incipient stage of many diseases), weakness, anemia, myopathy	3
<i>Lateral</i> , opisthotonos back, unable to right self to sternal	Moderate to marked depression	4-5
<i>Lateral</i> , flaccid paralysis, nonresponsive to stimuli	Head and neck trauma, tick paralysis, rabies, enterotoxemia	5
<i>Lateral</i> , twitching, seizures, forced running	Encephalitis, hepatopathy, head/neck trauma, rabies	5

*For owners and veterinarians: 1 = normal; 5 = grave.

the humming must be evaluated within the context of the existing situation. As an example, if a cria has recently been weaned, it is normal behavior for either the mother or the juvenile to hum excessively. If the same female were to change humming patterns for no apparent social change, more attention should be given.

Groaning is a vocalization generally associated with discomfort or pain, but it may be an acceptable sound

in a recumbent female in advanced pregnancy or during parturition. At other times, groaning may be associated with obstruction of the gastrointestinal or urogenital system. People who experience abdominal discomfort can relate to groaning.

Although not a vocalization, *grinding of the teeth* is an oral sound indicative of abdominal discomfort (colic). Such grinding is often accompanied by a pained

countenance, manifested by rigid facial muscles often described as a “fixed” facial expression.

Pouting is a normal consequence of an unpleasant interaction between SAC males and is typical after a fighting episode. The facial muscles become tense. The lower eyelid is pulled ventrally, exposing an area of conjunctival mucous membrane. The ears are positioned behind the vertical, the degree of flattening commensurate with the anger of the individual. The other prominent characteristic of pouting is the mouth held open and the nostrils flared, as if having difficulty breathing. SACs primarily breathe through the nares, and unless respiration is severely compromised, open-mouth breathing does not occur. Pouting animals are generally not in respiratory distress, so differentiating between pouting and dyspnea should be easy.

Although pouting is only performed by males, females that have been through a mutual spitting episode may stand with the mouth open and the lower jaw relaxed, as if to air out the mouth. When a muzzle or a spit rag is used to discourage a persistent spitter, it becomes evident that camelids dislike the smell the same as do humans.

Eyes

The large expressive eyes of SACs immediately attract attention, and it has been said that the eye is the window to the emotional state of an animal. Much may be learned by attentive evaluation of the eye (eyeball, eyelids). Observant people are quick to perceive a person's emotional state or illness on the basis of eye clarity, pupillary dilation or constriction, and eyelid position. Often a mother has only to look into the eyes of a child to sense excitement, apathy, depression, or guilt of a misdeed. The eyes of healthy or ill animals are also revealing. It is difficult to describe an apathetic look or a pained expression, but these are present in animals as well as humans.

The eyes of a healthy SAC should be clear and bright. The pupils should respond quickly to extra light. Poking a finger toward the eye should produce a blink reflex. The appearance of the eyes provides the basis for abnormal countenance (facial expression).

The cornea of an animal that is ill may appear to be glazed or cloudy. The pupil may be slow or nonresponsive to light, and the animal may not respond to a finger poked toward the eye. A SAC may have an inattentive gaze (“star gazing”). Closed eyelids indicate pain associated with the eye. Some systemic as well as local diseases stimulate excessive tearing or discharge.

ELEPHANTS

Normal Behavior

Offense and Defense

No one except a trained, qualified elephant handler should approach, come in contact with, or command an elephant. Elephants generally will not listen to or follow the commands of a stranger.

Elephants use several methods for offense and defense, including biting, slapping with the trunk, and grasping with the trunk and pulling, pushing, or throwing. As an offensive or defensive weapon, the trunk is without equal in the animal world. Handlers must appreciate the reach of the trunk and know the danger from the trunk of an angry elephant.

Elephants may purposely step on a person's foot. They are adept at kicking and may easily balance on one front and one hind leg. Extreme aggression may be exhibited by the elephant kneeling and head pressing on what they perceive as a threat, an inconvenience, or a toy. Even an animal in an elephant restraint device or on tethers may injure a person unfamiliar with an elephant's reach or its signals of aggressive intent.

Although the swinging tail is usually not considered an offensive weapon, it must be considered when administering medication in the rear quarters or when tethering a hind leg. Being soundly struck is painful, and a blow to the face or head may be injurious.

These warnings are provided not to frighten veterinarians or handlers, but rather to impress on them that these normally gentle giants are so large and so strong that serious injury or death may result from improper assessment of an elephant's behavior.

Communication

Vocalization. More than 30 vocalizations have been distinguished in African elephants, but only 10 have been studied in Asian elephants. Elephants may produce an infrasonic sound that is not audible to humans. This sound is used in long-distance (several kilometers) communication and may be important in family communications and for females to advertise receptivity, but not of consequence to this discussion. Vocalizations to be aware of during restraint procedures include the following:

- **Bellow:** A loud fear- or pain-related call.
- **Blow:** An audible air blast from the trunk, or a visual blast containing dust or food particles.

- **Scream:** Produced when an elephant is extremely excited or angry.
- **Trumpet:** Loud, high-frequency, pulsating sound.
- **Musth rumble:** A deep-throated, guttural or bubbly vocalization that is loud and low.
- **Trunk tapping:** Elephants may amuse themselves or exhibit slight irritation by tapping on a smooth, flat surface with the tip of the trunk, producing a hollow, thumping sound.

Musth is a normal periodic behavior in mature male elephants. For safety, handlers must recognize the primary signs of musth, including aggressive behavior, drainage from the temporal glands, dribbling urine from the prepuce, and unusual vocalization (“musth rumble”). Other signs that are not unique to musth but often occur during musth are anorexia, dehydration, and somnolence. A bull elephant in musth is dangerous and should be handled only from behind a protective barrier.

Body language. All personnel working with elephants should understand basic elephant body language behaviors.²⁶ Particular attention should be paid to the ears and trunk to assess the mood of an elephant (Figures 4-13 and 4-14). The elephant is unique by possessing a highly mobile trunk that has many important functions, including eating, drinking, breathing, lifting, vocalizing, performing social discipline, and manipulating tools. Furthermore, the trunk is used to deliver volatile and nonvolatile odorants to olfactory center receptors in a specialized nasal cavity and cribiform plate. Trunk position becomes an important consideration in the subsequent discussion of elephant behavior.

Behaviors to be aware of include the following²²:

- **Alert:** The elephant stands facing a person with the head raised, ears spread, tail raised, and trunk raised or turned in a “sniff” position.
- **Wariness:** The elephant is in heightened alertness, and with eyes wide open, glances at other elephants.
- **Sniff:** The trunk is extended down and forward in a J shape, with the tip out horizontally to sniff another elephant or a person.
- **Mock charge:** The elephant runs toward another elephant or a person with ears extended, head and tusks held high, tail elevated or not elevated, and trunk extended. The charging elephant stops before reaching the target and usually trumpets.
- **Real charge:** The trunk is tucked under the head, and the head is up and attempts to contact the



Fig 4-13 Elephant relaxed. (See Color Plate 4-13.)



Fig 4-14 Elephant alert and threatening. (See Color Plate 4-14.)

target. The ears are usually close to the head, and usually there is no trumpeting.

- **Slap:** An elephant strikes another elephant with the trunk.
- **Kick:** An elephant may strike forward with a forelimb or toward the side or rearward with a hind limb.

All these behaviors are noteworthy because persons may be severely injured if these behaviors are directed toward them.

Facial expression. Elephants have small eyes, and facial expressions are overshadowed by ear and trunk movements; however, the degree of alertness is indicated by the openness of the eyelids. Eyes should be clear and bright. Elephants do not have tear ducts, so excess lacrimal secretion flows from the conjunctival sac and down the cheek. It is important to differentiate this clear fluid from the opaque and discolored drainage associated with conjunctivitis or keratitis.

Social Behavior

In the free-ranging state, both Asian and African female elephants exist in family groups of 8 to 12 individuals. The adult females and their offspring are closely related to the matriarch, who is usually the oldest female.

The males live in bachelor herds or are solitary. Adult males only interrelate with the family when a female is in estrus. Adolescent males leave their birth herd as teenagers and enter into bachelor herds. Constant sparring occurs as they test their physical prowess in anticipation of becoming a breeding bull. Size and age are the determinants of dominance in males. Males in musth are dominant over nonmusth males.

Neonatal calves and juvenile elephants are given constant care by their mother and *allomothers* (other females in the herd, sometimes referred to as “aunts”). Elephants have an extended adolescence similar to that of humans. Schooling is constant to provide proper behavior within the family group and to develop survival skills such as foraging and predator avoidance.

Historically, captive elephants have not often been in situations where they can form a family group; however, revised guidelines from the Association of Zoos and Aquariums and other groups are addressing this issue. The most dominant elephant in a captive herd is usually the largest and oldest female. Some elephants may become incompatible with other individuals.

Interaction with Other Animals

Free-ranging elephants are dominant to other animals in their habitat. Adult elephants are not subject to predation, but infants and juveniles may be preyed on if they wander away from a herd. Captive elephants are generally exhibited in smaller groups, but they may be trained in circuses to allow close association with horses, dogs, and even tigers.

Hierarchic Status

In free-ranging family groups, the matriarch is the dominant individual. All elephants within the group have a definite place within the family. Likewise, in bachelor herds of males there is also a hierarchic arrangement.

In captivity, elephants kept in groups also establish a dominance hierarchy. New elephants must be introduced to a herd slowly to avoid undue trauma. When a group of elephants have been maintained together for a long time, the casual observer may not be aware of dominance.

Two elephants kept together at a small California zoo got along quite amicably for years. Finally the younger, beta animal asserted herself and a fight ensued, the beta animal knocking the alpha female down. Subsequently it was determined that the alpha female had developed severe polyarthrititis and had lost her ability to maintain her dominance.

Locomotion

Free-ranging African elephants may cover considerable distance daily in search of food (distances are shorter in environments that more readily supply their needs [i.e., richer in food and water sources]). The basic gait is a walk, which may be carried out swiftly and silently (15.3 kph, or 4.5 mph). The faster gait is an *amble* (modified pace), with the left hind foot hitting the ground, followed by the left fore foot, then the right hind and the right fore foot.¹⁵ Elephants do not trot or gallop, but one should not underestimate their speed and agility potential. The maximum speed of an elephant has been determined to be 24 kph (15 mph), not 40 kph (25 mph), as has been reported in the popular literature. This is faster than all but the fastest human runners are capable of attaining.

Food Intake

Elephants are opportunistic herbivores, browsing when expedient, and likewise grazing if that is the forage available. Forage is grasped with the trunk and inserted into the mouth. Elephant caretakers should spend considerable time observing the eating behavior of each animal under their charge. Changes in the manipulation of forage with the trunk may be a key factor in diagnosing incipient illness. Inappetence is one of the first responses of most animals to illness.¹⁸

Defecation and Urination

Five to eight large fecal boluses are passed, 14 to 18 times a day. Each bolus may weigh up to 2 kg (4.4 lb). The daily quantity produced varies with the size of the animal and its food intake but may reach 110 kg (240 lb). The color of the freshly passed feces varies somewhat with the forage consumed but is generally greenish brown, darkening with time and exposure to air. Boluses should break apart easily.

Adult elephants may produce 40 to 60 L (10.5-15.9 gal) of urine daily. On average, each individual discharge is 5.5 L (1.45 gal). The color is pale yellow and has little odor. Urine is slightly turbid.

Scent Communication

Elephants obtain scent information from the environment using two different modalities. Air inhaled through the trunk is carried to receptors in a specialized nasal cavity and cribriform plate.²⁴ Air entering the distal trunk is warmed to enhance volatilization of odorants before reaching the olfactory area. An elephant may determine the direction an odor is coming from by manipulating the trunk into a sniff position (testing the air).

The trunk is also used to deliver less volatile chemicals (pheromones) to the vomeronasal organ by dipping the tip of the trunk into a freshly passed puddle of urine containing the pheromones. Droplets of urine on the tip of the trunk are transported to the roof of the mouth near the orifice of ducts leading to the vomeronasal organ. This assists breeding males in detecting when a female is in estrus.

The temporal (musth) gland is a modified apocrine sweat gland that is located caudal to the lateral canthus of the eye. Under specific circumstances the gland discharges both secretions from the gland itself and ultrafiltrates from blood. Breeding males in musth secrete copious quantities of a malodorous, brownish liquid that flows down the side of the face. The secretion from low-ranked males in a bachelor herd has a mellow, honeylike odor, which does not raise the ire of breeding bulls. Females may also produce temporal gland secretion when excited, stressed, or angry or when pregnant and during parturition.²⁴ Assessment of swelling and secretion from the temporal gland is crucial to evaluation of behavior.

Recumbency

Elephants do not rest comfortably in sternal recumbency. Pressure on the abdomen exerts visceral pressure forward and prevents the diaphragm from effectively participating in respiration (and elephants lack an effective pleural space that allows lung movement in that position). However, most elephants will sleep in lateral recumbency for a few hours each night and even at rest during the day. When lying down, the elephant sits down on one hind leg, with the front legs extended forward (stretch position). It then lowers the body to the floor and rolls over onto its side. When arising, the first action is to rock the upper fore and hind legs forward and backward to give momentum for lifting the body to the stretch position. The front limbs are then straightened, followed by each hind limb.

Reproductive Behavior

Courting. A breeding bull may pick up pheromone scent of a female in estrus by sniffing at deposited urine. He may then enter a family group and sniff females until he finds the estrous female. He may follow the female if she is not in full estrus, until she stands.

Copulation. Copulation is in the standing position. The extended penis of the male must manipulate the vulva located on the caudal abdomen, lifting the urogenital sinus to a position where the male may thrust into the pelvic vagina. The distal penis may be bent into a hook by the special anatomic apparatus of the retractor penis muscles, which insert near the glans penis.

Behavioral Changes Associated with Illness

The general statements about abnormal behavior in camelids are also applicable to elephants. Specifically, behavioral changes in elephants associated with illness include listlessness, decreased movement, and depression. Inappetence is common. Deviations from normal behavior may be subtle but are critical to proper detection of incipient illness. Trunk, tail, and ear movement should be observed. Ear movement of a depressed elephant is slowed and in severe depression may cease. Likewise, tail movement slows and ceases. Often the trunk relaxes and may dangle to rest on the ground. Normal investigative touching with the trunk may become incoordinated or may cease. The head may be positioned in a relaxed attitude.

Abdominal pain may be evidenced by peculiar body positions, kicking at the abdomen, and straining during defecation or urination. Decreased or increased (diarrhea) fecal output may be an early sign of impending illness, as may the character of the feces or urine. A shiny coating on individual fecal boluses ("wrapped in cellophane") indicates constipation. The feces of an excited or angry elephant may quickly become softer.

A change in the gait may indicate weakness, a central nervous system disorder, or lameness. Lameness may be caused by pain when pressure is applied to an inflamed structure (supporting-leg lameness) or when moving a limb (swinging-leg lameness). Nonpainful conditions may also cause an altered gait (ankylosis, conformation defects, angular limb deviation).

Lameness diagnosis in elephants is discussed here to encourage recognition of subtle changes in gait that

portend more serious problems. The basic principles of lameness diagnosis used for horses may also be applied to elephants, except that the elephant does not lift its head when a sore forelimb strikes the ground. The examiner must rely on the time a foot remains on the ground.

STRESS

Stress is the cumulative response of an animal to interaction with its environment through receptors,² or “the biological response elicited when an animal perceives a threat to its homeostasis.”²⁰ The threat is a stressor (stress-producing factor), and it is important to recognize that a psychologic perception of a threat may be as important as the response to a physical stressor.

The biologic responses to stress are adaptive, directed at coping with environmental change, and every animal is subject to stress, whether free-ranging or in captivity. Intense or prolonged stimulation may induce detrimental responses (distress).²⁰

Species vary in their perception of a threat and how they process the information received to evoke a physiologic response. Using any single laboratory parameter to determine the stress status of an animal is unreliable.

Little significant research has been conducted of stress in camelids and elephants, so the following observations are based on research in other animals. We should not assume that camelids, elephants, or other wild animals do not become distressed.

A *stressor* is any stimulus that elicits a biologic response when perceived by an animal. Some potential stressors acting on animals are listed next so that you will consider these important factors when handling wild animals. *Somatic* stressors (stimulation of the physical senses) include temperature changes, strange sights, unfamiliar sounds, touches, odors, thirst, and hunger. *Psychologic* stressors include anxiety, fright, terror, anger, rage, and frustration. Closely allied are *behavioral* stressors, including overcrowding, lack of social contact, unfamiliar surroundings, transport (circus elephants may become accustomed to transport), and lack of appropriate foods. Miscellaneous stressors include malnutrition, toxins, parasites, infectious agents, burns, surgery, and drugs.

It is becoming increasingly important to recognize that stimulation of visual and auditory senses has a marked bearing on cumulative stress. Modern interpretation makes no distinction between specific and nonspecific responses, because there is marked species variation in how organisms process and act on

stimuli.²¹ An individual may even display varying responses, depending on which stimuli are acting on it at a given time and the experience, hierarchic status, nutrition status, and history of a previous adaptation to the stimulus.^{19,21}

Body Response to Stress Stimulation

The central nervous system (CNS) receives messages from receptors, processes the information, and initiates a biologic response through one or more of the following pathways: behavior, autonomic nervous system, neuroendocrine system, or the immune system^{9,19} (Figure 4-15).

Animals respond in appropriate ways to stimulation of specific receptors. For example, when cold receptors are stimulated, the body experiences a sensation of coolness, and various somatic and behavioral changes occur that conserve heat and stimulate increased heat production. The animal is adjusting to a new situation (*homeostatic accommodation*). If heat is the stressor, the animal tries to take steps to cool itself.

The autonomic nervous system (ANS) deals with short-term stress responses (“flight or fight” scenario), although any tissue innervated by autonomic nerves may be affected (e.g., increased peristalsis). The ANS is seldom a factor in distress because the duration of stimulation is short.

The neuroendocrine system is a major pathway that mediates the development of signs of distress. Often this pathway is thought to be the hypothalamic-pituitary-adrenocortical (HPA) pathway (Figure 4-15). However, modern research has conclusively demonstrated that all systems modulated by the hypothalamic-pituitary axis may be affected (growth, reproduction, immunity, metabolism, behavior).^{19,21}

Individual animals and species vary in the primary pathway used to cope with change. The pathways used by the elephant are unknown. However, continuous adrenocortical stimulation and excessive production of cortisol elicit many adverse metabolic responses. Psychologic as well as physical changes may occur. The clinical syndromes of adrenocortical stimulation have been identified in some species (human, dog, horse, laboratory animals). There is much still to learn about the effects of hypercorticism in wild animals. However, the basic biologic effects of cortisol should be understood.^{2,3,9}

For example, protein catabolism and lipolysis contribute to the pool for glyconeogenesis. Slight to moderate hyperglycemia has a diuretic effect, producing polyuria and polydipsia. Prolonged hyperglycemia

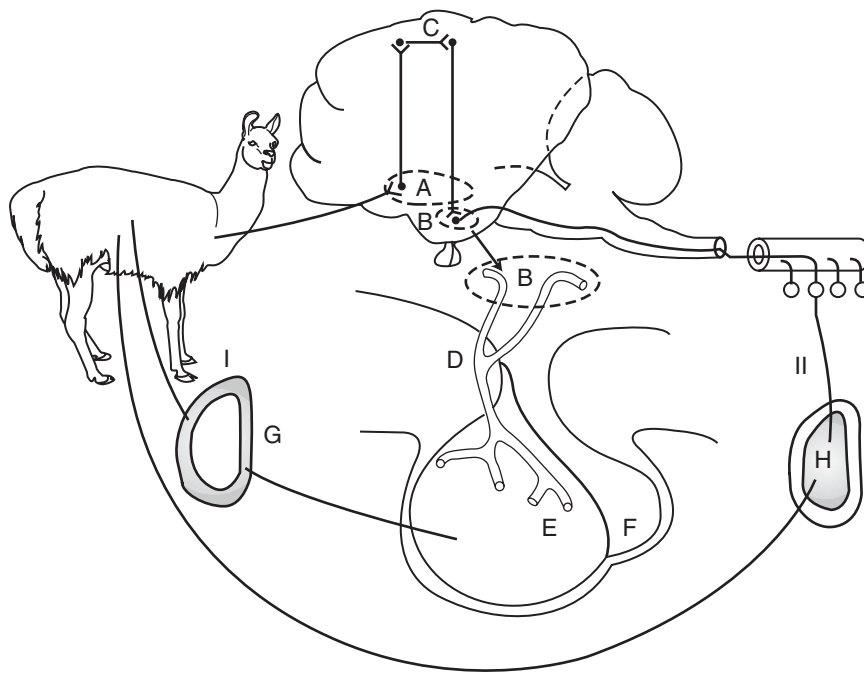


Fig 4-15 Diagram of the neuroendocrine pathways of stress. *I*, Hypothalamic-pituitary-adrenocortical pathway; *II*, alarm reaction (adrenal medulla); *A*, thalamus; *B*, hypothalamus; *C*, cerebral cortex; *D*, hypothalamus/pituitary portal vein; *E*, anterior pituitary; *F*, posterior pituitary; *G*, adrenal cortex; *H*, adrenal medulla.

stimulates the beta cells of the pancreas to produce more insulin.

Cortisol reduces the heat, pain, and swelling associated with the inflammatory response, an effect useful in the treatment of many diseases. The antiinflammatory action of cortisol is produced by reducing capillary endothelial swelling, thus diminishing capillary permeability. Additionally, capillary blood flow is decreased by the action of cortisol. Both these actions are helpful in shock therapy.

The integrity of lysosomal membranes is enhanced by cortisol. Under such circumstances, bacteria and other particulate matter are engulfed by phagocytes, but hydrolytic enzymes (which would destroy the organisms) are not released from the lysosomes.

Within a few hours of a cortisol stress response, there is a reduction in the number of circulating lymphocytes ($\geq 50\%$). Lymphocyte levels return to normal within 24 to 48 hours after cessation of stress. The effect of stress on the total leukocyte count varies with the species and depends on the normal relative leukocyte distribution. Species with normally high percentages of lymphocytes, such as mice, rabbits, chickens, and cattle, respond with a lymphopenia and neutrophilia and a decrease in total leukocytes. Dogs, cats, horses, and humans, having relatively low lymphocyte counts, respond with an increase in leukocytes.²

Elephants generally have a slightly higher percentage of lymphocytes than neutrophils, but the numbers are close enough that it is difficult to identify a stress hemogram in an elephant.

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Color Plate 4-1 Male camelids fighting, chest butting. (For text mention, see Chapter 4, p. 34.)



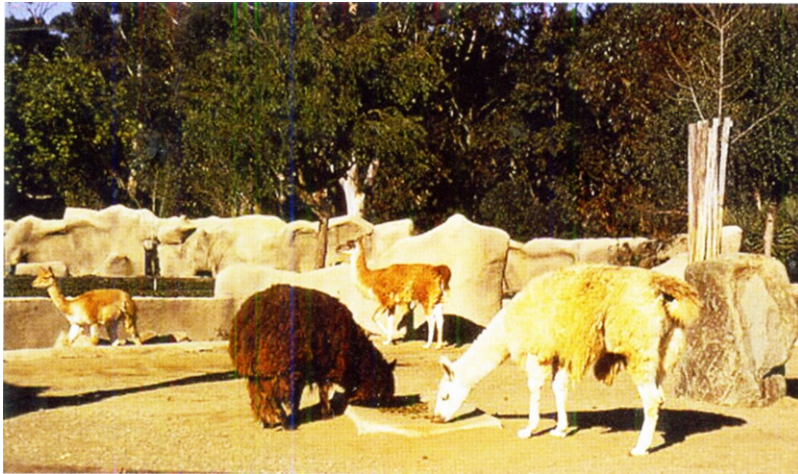
Color Plate 4-2 Male camelids fighting. (For text mention, see Chapter 4, p. 34.)



Color Plate 4-5 Ear position: alert in back, mild aggression in front. (For text mention, see Chapter 4, p. 36.)



Color Plate 4-6 Submissive crouch. (For text mention, see Chapter 4, p. 37.)



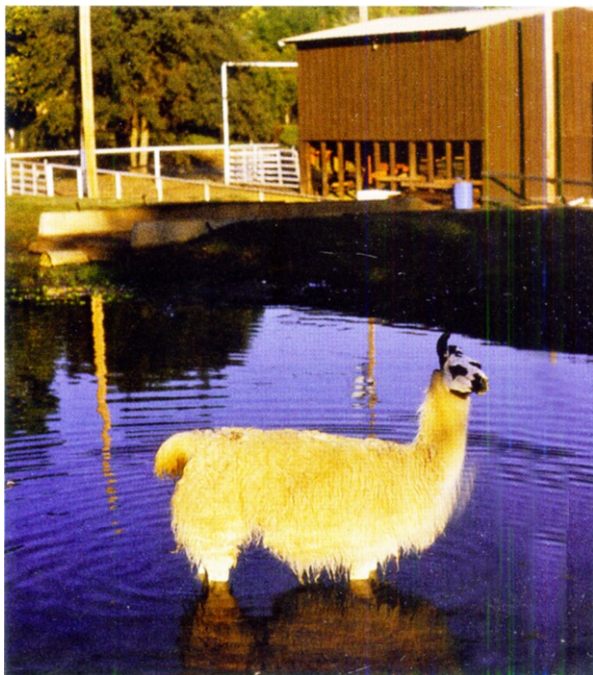
Color Plate 4-7 Four South American camelids: *top left*, vicuña; *top right*, guanaco; *bottom left*, alpaca; *bottom right*, llama. (For text mention, see Chapter 4, p. 37.)



Color Plate 4-8 Llama defecating. (For text mention, see Chapter 4, p. 38.)



Color Plate 4-9 Llama dusting. (For text mention, see Chapter 4, p. 38.)



Color Plate 4-10 Llama standing in a pond to cool off. (For text mention, see Chapter 4, p. 39.)



Color Plate 4-11 Sternal recumbency with front legs forward. (For text mention, see Chapter 4, p. 40.)



Color Plate 4-13 Elephant relaxed. (For text mention, see Chapter 4, p. 44.)



Color Plate 4-14 Elephant alert and threatening. (For text mention, see Chapter 4, p. 44.)

CHAPTER 5

Ionophores: Salinomycin Toxicity in Camelids

DAVID E. ANDERSON

MECHANISM OF ACTION

Ionophores are a class of naturally occurring antimicrobial drugs with a wide range of activity. These polyether acid ionophore antimicrobials are produced by the Actinomycetales order of filamentous bacteria. These drugs are widely used to control or prevent coccidian parasite infestation in the intestinal tracts of poultry, cattle, and pigs. Ionophores have a beneficial side effect of helping to maintain balance in the intestinal bacterial populations during periods of high-volume concentrate (e.g., corn, grain) feeding. This effect results in a more efficient feed-to-body weight gain ratio. Greater feed efficiency translates to improved economic returns for the meat producer.

These drugs are referred to as *ionophores* because their mechanism of action involves interference with ion exchanges at the cell membrane. Specifically, these drugs are open-chained oxygenated heterocyclic rings with terminal carboxyl groups. This structure allows the drugs to form lipid-soluble complexes with ions such as calcium (Ca^{++}), potassium (K^+), sodium (Na^+), and magnesium (Mg^{++}). These complexes cause ion fluxes independent of ion channels and membrane potentials, causing decreased adenosine triphosphate (ATP) production, increased ATP utilization, and eventually cell death.

Salinomycin has greatest affinity for rubidium (Rb^+) $> \text{Na}^+ > \text{K}^+ \gg \text{cesium} (\text{Cs}^+)$, Mg^{++} , Ca^{++} , and strontium (Sr).³ Salinomycin induces a pH-dependent disruption of cellular respiration and mitochondrial damage, ultimately resulting in cell death.

These drugs have a particular predilection for striated muscle cells because of the high concentration of calcium ions and extreme activity involving movement of ions. Thus, overdosage of ionophores most often causes clinical signs associated with skeletal muscle dysfunction and myocardial dysfunction.⁵

ETIOLOGY AND EPIDEMIOLOGY

Intoxication with ionophores through accidental overdosage has been reported in the target species for which the products are approved, as well as a wide variety of nontarget species (e.g., horses, cattle, sheep, turkeys, pigs, dogs, cats, rabbits, white-tailed deer, guinea fowl, ostriches, chickens, camels, alpacas).^{5,6} Differential species sensitivity to ionophores has been recognized and varies with each ionophore. The ionophores most frequently involved in accidental toxicoses are monensin, lasalosid, and salinomycin (Table 5-1).

Most often, poisonings are caused by feed-mixing errors, accidental contamination of feed, or approved products being fed to inappropriate species. Toxicity may occur from ingestion of single large dosages or from multiple feedings of smaller dosages. Ionophores have variable bioavailability but wide volume of distribution in the body. These antimicrobials are metabolized in the liver by glutathione and cytochrome P-450 enzyme pathways. Ionophores are rapidly eliminated by biliary secretion with no renal clearance; thus postmortem analysis of tissues, urine, and intestinal contents usually is unrewarding. Age may have some effect on sensitivity to ionophores. Adult turkeys are more susceptible to toxicity than young stock.⁷ Young turkeys may tolerate continuous feeding of 40 parts per million (ppm) or more, but adults may succumb to feeding rates as low as 15 ppm. A number of drug interactions have been identified with ionophores. Salinomycin toxicity is increased when fed in combination with dihydroquinolone antioxidants or tiamulin.

Environmental impact of ionophores is not well described. Ionophores are absorbed rapidly from the gastrointestinal (GI) tract and quickly metabolized by the liver and GI tract. Elimination half-life is approximately 2 to 3 hours, and more than 90% of the drug is excreted in the feces within 48 to 72 hours of ingestion.

Table 5-1**Comparison of Differential Species Sensitivity to Toxicity of Common Ionophores (mg/kg Body Weight)**

Species	Monensin	Lasalosid	Salinomycin
Horse	2-3	21.5	0.6
Cow	20-80	50-150	
Sheep	12	75-350	
Chicken	200	71.5	44.3
Pig	16-50	30-50	
Camel	No data	No data	0.5-1.5
Alpaca/llama	No data	No data	0.5-1.5 (estimate)

Salinomycin breaks down in feces over 21 days. Ingestion of contaminated feces in amounts required to intoxicate an animal is extraordinarily unlikely.

CLINICAL SIGNS

Clinical signs of ionophore toxicity vary among species affected^{1,3,5,6} (Box 5-1). Clinical signs are dose dependent and arise from the dominant organ system involved in each species, including musculoskeletal, cardiopulmonary, neurologic, and smooth muscle, or acute death may occur without clinical signs. Thus, any combination of anorexia, depression, muscle tremors, weakness, incoordination, ataxia, dyspnea, diarrhea, congestive heart failure, exercise intolerance, unthriftiness, and death may be seen. Although effects on fertility and fetal development are scant in mammalian species, lost egg production, decreased fertility, early embryonic death, deformed yolk, and weak newborn chicks have been observed after intoxication of laying hens.³ Interestingly, sows fed 40 to 60 mg salinomycin/kg feed demonstrated improved number of piglets born alive, higher piglet body weight, and sow weight gain during gestation.²

A group of 120 dromedary camels received one feeding of 2 to 4 kg each of a pelleted diet that had been accidentally contaminated with 138.9 ppm salinomycin.⁸ This translates to a dosage of salinomycin between 0.7 and 1.4 mg/kg body weight. Within 24 hours, 25 camels had weakness and incoordination, which progressed to recumbency within 4 to 6 hours. Within 48 hours, 50 camels were affected, and one had died. Subcutaneous edema and myoglobinuria were noted. Camels demonstrated excessive lacrimation and stiff gait. Over 10 weeks, all 120 camels showed

Box 5-1**Clinical Signs Observed in Various Species with Ionophore Toxicity****Horses**

Anorexia, sweating, abdominal pain, apparent depression, incoordinated walking, rapid heart rate and arrhythmias, recumbency and death

Cattle

Anorexia, diarrhea, apparent depression, difficulty breathing, incoordination, tremors, recumbency and death

Poultry

Anorexia, diarrhea, weak vocalizations, incoordination, limp position of wings and abducted limbs, decreased egg production

Dogs

Incoordination, weakness, difficulty breathing, difficulty urinating, apparent depression, constipation

Camels

Weakness, hind limb incoordination, death

Alpaca/Llama

Decreased anal and tail tone, weakness, trembling, apparent depression, incoordination, difficulty urinating, difficulty breathing, death

clinical signs, and 58 died (i.e., 100% morbidity; 48% mortality). Most (80%) of deaths occurred within 2 weeks of the feed contamination. The last recorded death occurred 5 weeks after the single ingestion of salinomycin.

Of the 62 surviving camels, 30 remained ambulatory throughout, and 32 became recumbent and required up to 10 weeks to regain voluntary ambulation. Interestingly, only 3 of 58 calves (5.2%) 7 months old or less died despite being clinically affected. In comparison, 55 of 62 (89%) adults died. Camels were immediately treated with a combination of selenium, vitamin E, dexamethasone, vitamin B₁, and oral cathartics. Severe cases received intravenous (IV) fluids and diuretics. Serum biochemistry and hematology revealed leukocytosis, neutrophilia, hyperphosphatemia (mean, 14.5 mg/dL), hypocalcemia (mean, 9.2 mg/dL), and marked increases in creatine kinase (CK; mean, 58,333 U/L), lactate dehydrogenase (LDH; mean, 4044 U/L), and aspartate transaminase (AST; mean, 1209 U/L). Necropsy examination found skeletal muscle edema, pale streaking of myocardium, fatty liver disease, and hemorrhagic enteritis.

In a pilot study of four young camels, two served as controls, with a diet of 138 ppm salinomycin fed to one and a diet containing 20 ppm of monensin fed to the other. The salinomycin induced anorexia, ataxia, and difficulty rising within 36 hours. The monensin caused anorexia within 36 hours and difficulty rising 72 hours after feeding. In the salinomycin camel, CK rose to 2580 U/L at 48 hours and 146,000 U/L after 8 days. The monensin-fed camel had CK of 459 U/L on day 5 and 53,900 U/L on day 8. This accidental poisoning and toxicity trial suggest that camels are sensitive to salinomycin in the range of 70 to 105 mg/kg body weight as a single dose. No long-term follow-up was available to assess survival or fertility effects.

In 2003, a death and illness outbreak was observed in approximately 10 herds of alpacas in the north-central region of Ohio.⁴ Several farms of alpacas, Huacaya and Suri breeds, were fed a diet that had been accidentally contaminated with salinomycin at a concentration of 60 to 90 ppm. Alpacas had been offered the diet at a rate of approximately 0.25 to 0.5 kg per head per day for 3 to 5 days (daily dosage, ~0.5-1.5 mg/kg body weight; accumulated dosage, ~1.5-7 mg/kg). Alpacas demonstrated rhabdomyolysis, weak tail tone, incoordination, and difficulty urinating beginning on day 3 of feeding. By day 4, acute deaths were noted, and continued for at least 21 days after discontinuation of contaminated feed. During the first 5 days of the clinical period (days 4 through 8 after initiating feed; days 1 through 5 after removing feed), acute rhabdomyolysis and acute death were the dominant clinical syndrome. These alpacas developed muscle tremors, weakness, ataxia, exercise intolerance, diarrhea, anorexia, depression, recumbency, or acute death. After day 5 of treatment, myocardial injury, cardiopulmonary failure, and death were the dominant syndrome. These alpacas demonstrated weakness, nasal flaring, dyspnea, tachypnea, anorexia, exercise intolerance, and acute death. Deaths associated with cardiac disease (myocardial fibrosis) were seen up to 2.5 years after the feed ingestion.

DIAGNOSIS

Antemortem Diagnosis

Diagnosis of ionophore toxicity is based on clinical signs, historical data, clinicopathologic data, necropsy data, and analysis of feed. Ionophore drugs may not be reliably found in body tissues, blood, intestinal contents, or feces. Occasionally, analysis of rumen contents or feces yields positive tests, but diagnosis is most consistently made by analysis of suspect feed. Other causes of clinical signs should not be dismissed

until a definitive diagnosis is made. Analysis of the feed given immediately before onset of clinical signs (e.g., within the previous 5 days) should be analyzed for ionophores and other contaminants. Several bags of feed should be analyzed to account for variations in mixing.

Electrocardiography (ECG) and echocardiography may provide information regarding myocardial function. Characteristic ECG changes include increased S wave, depression of ST segment, increased T wave, absent P wave, prolonged QT interval, premature ventricular contractions, arrhythmias, atrioventricular (A-V) block, atrial fibrillation, and ventricular fibrillation.³ Echocardiography changes may include changes in fractional shortening. These tests are indicators of severity of myocardial injury and may not be used to rule out myocardial disease.

Clinical Pathology

Depending on severity and dehydration, increased serum concentrations of alkaline phosphatase (ALP), AST, LDH, SDH, gamma-glutamyltransferase (GGT), creatine phosphokinase (CPK, CK), chromium (Cr), bilirubin, blood urea nitrogen (BUN), glucose, hematocrit (HCT), and phosphorus (P) may be observed, while decreases in Ca, K, and Na are occasionally found (Box 5-2). In many acute cases of ionophore toxicosis, CK enzyme activity in serum is extremely elevated, often exceeding 100,000 units/mL serum. AST enzyme

Box 5-2

Possible Serum Biochemistry and Hematology Changes with Ionophore Toxicosis

Serum Biochemistry

Increases

Creatine kinase (CK)
Aspartate transaminase (AST)
Lactate dehydrogenase (LDH)
Alkaline phosphatase (ALP)
Bilirubin
Blood urea nitrogen (BUN)

Decreases

Calcium
Potassium

Hematology

Hemoconcentration

Increased packed cell volume (PCV)
Increased total platelets (TP)

Box 5-3**Select Differential Diagnoses to Consider with Suspected Ionophore Toxicosis****Horses**

Colic: intestinal accidents, blister beetle ingestion, exertional rhabdomyolysis

Poultry

Nutritional myopathy, plant toxicity (e.g., coffee senna), botulism, salt toxicity, mycotoxins, viral arthritis

Cattle

Vitamin E deficiency, selenium deficiency, selenium toxicity, plant intoxication (e.g., coffee senna, coyotillo, white snakeroot), clostridial myositis

Alpaca/Llama/Camel

Vitamin E deficiency, selenium deficiency, selenium toxicity, plant intoxication, clostridial myositis, meningeal worm, *Sarcocystis* myopathy

activity increases lag behind CK increases by 24 to 72 hours. Liver-specific enzymes may increase because of ionophore effects on liver cells or because of secondary metabolic hepatopathy. Serum troponin I concentration increases based on severity of myocardial injury and ranges from less than 0.15 up to 44 ng/mL (normal, <0.2 ng/mL).

Differential Diagnoses

Differential diagnoses are highly variable depending on clinical signs and species affected (Box 5-3). In cases in which rhabdomyositis is present (e.g., extreme increase in serum CK enzyme activity), clostridial myositis, selenium toxicity or deficiency, vitamin E deficiency, mycotoxins, or severe muscle trauma (e.g., capture myopathy, compartment syndrome) should be investigated. The diagnosis is supported by the moderate morbidity and high mortality characteristic of ionophore poisoning.

Postmortem Diagnosis

Clinical diagnosis should be reinforced by necropsy examination findings, as determined by dose and duration of exposure before death (Box 5-4). In acute death soon after exposure, necropsy yields no obvious lesions. After 24 to 48 hours, examination of the cells by electron microscopy may reveal mitochondrial swelling as the earliest lesions.

Box 5-4**Typical Gross and Histopathologic Findings with Ionophore Toxicosis****Gross Pathology**

Pale skeletal muscle

Pale cardiac muscle

Dilated ventricles

Petechiation

Ecchymosis

Yellow to white streaks in myocardium

- Horses: predominantly cardiac muscle
- Sheep, swine, dogs: predominantly skeletal muscle
- Cattle, poultry: equal prevalence of skeletal and cardiac muscle
- Alpacas and camels: cardiovascular lesions seem more common, but severe rhabdomyolysis has been seen.

Histopathologic Findings

Focal myocyte degeneration

Myocardium: vacuolation, swelling, eosinophilic staining

Electron microscopy: swollen mitochondria, swollen sarcoplasmic reticulum, disruption of myofibrils

Myocardial cell damage replaced by scar tissue

As time progresses, myocardial injury and muscle cell injury are more obvious, with pale streaking of the muscle and histologic appearance of injury (Figure 5-1). Cell death is followed by inflammation, and finally, fibrosis consistent with muscle cell damage and necrosis. Heart lesions may include epicardial and endocardial hemorrhage, pale streaking of the myocardium, pericardial effusion, pleural effusion, peritoneal effusion, pulmonary congestion, subcutaneous edema, perirenal edema, hepatomegaly, splenomegaly, and pale streaking in skeletal muscles (Figure 5-2).

Ionophore toxicosis may be associated with rapid overgrowth of *Clostridium* spp., and hemorrhagic enteritis has been observed during necropsy of salinomycin-intoxicated alpacas. Histologic examination of heart tissues reveals myocardial swelling, vacuolization, hyaline degeneration, and fibrosis. Renal tubular and hepatocellular necrosis may be present. Skeletal muscle injury varies, as in the myocardium.

MANAGEMENT AND THERAPY

Treatment of ionophore toxicosis is difficult because no specific antidote exists. The first principle of treatment is to remove all sources of contaminated feedstuffs. Enteral treatments aimed to decrease absorption of drugs from the GI system may be administered (e.g., mineral oil, activated charcoal, cathartics), but

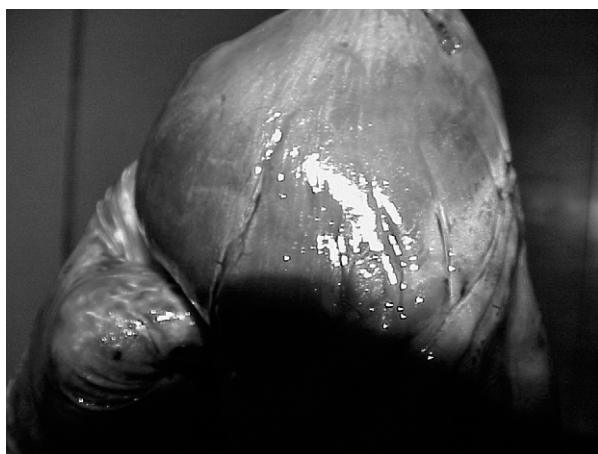


Fig 5-1 Pale streaks in heart muscle of alpaca that died 9 days after exposure to salinomycin-contaminated feed. (See Color Plate 5-1.)



Fig 5-2 Pale streaks in skeletal muscle of alpaca that died 9 days after exposure to salinomycin-contaminated feed. (See Color Plate 5-2.)

efficacy is questionable because of rapid absorption of the drug and delay in onset of clinical signs and recognition of intoxication for several days. Symptomatic treatments may be administered as supportive care when economically feasible (e.g., IV fluids, electrolytes as needed). When severe rhabdomyolysis is present, muscle relaxants (e.g., methocarbamol) and anxiolytic drugs (e.g., acepromazine) may be administered. Vitamin E and selenium therapy may aid in recovery by acting as an antioxidant. IV fluids are administered to prevent renal tubular damage from myoglobin depletion of oxygen. If clinical signs of pulmonary edema (seen as increased breathing effort) are present, diuretics (e.g., furosemide) and bronchodilators (e.g., aminophylline) may be used to ease cardiopulmonary strain.

LONG-TERM EFFECTS

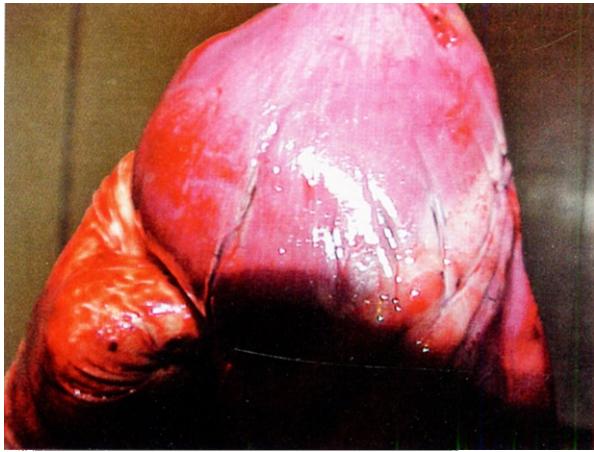
Clinical signs may not be observed for many months after ingestion of salinomycin. Chronic fatigue, sporadic deaths, and ill effects have been seen in camelids with heart muscle damage. The clinical significance of the salinomycin damage in long-term setting of health and fertility is not well characterized. The most common cause of death or debilitation after ionophore poisoning is myocardial injury. One alpaca died 2.5 years after ingestion of a feed containing 60 to 90 ppm salinomycin because of cardiac arrhythmias during a routine anesthesia. Necropsy revealed myocardial fibrosis, which may have caused the fatal arrhythmia.

PREVENTION

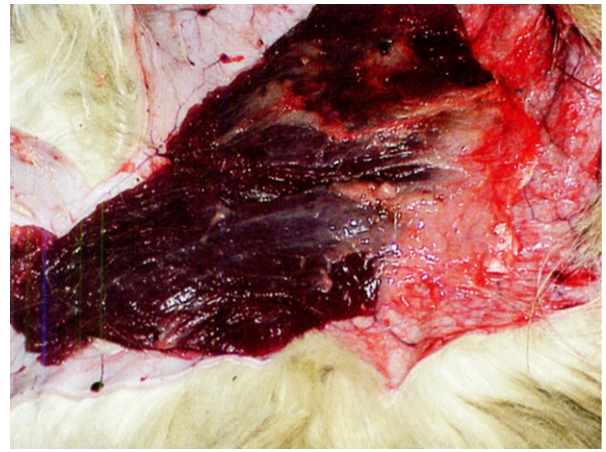
Ionophores should be used exclusively in the target species at the recommended amounts unless sufficient safety and efficacy studies warrant extralabel use. Feed mills should take extreme precautions to prevent accidental overdosage of feed or accidental contamination of inappropriate species feeds. Ideally, feeds intended for use in camelids, horses, and other high-risk species should be mixed at mills where no feed additives are used.

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Color Plate 5-1 Pale streaks in heart muscle of alpaca that died 9 days after exposure to salinomycin-contaminated feed. (For text mention, see Chapter 5, p. 54.)



Color Plate 5-2 Pale streaks in skeletal muscle of alpaca that died 9 days after exposure to salinomycin-contaminated feed. (For text mention, see Chapter 5, p. 54.)

CHAPTER 6

Emerging Diseases at the Interface of People, Domestic Animals, and Wildlife

ROBERT A. COOK AND WILLIAM B. KARESH

Increasingly, diseases are moving among people, domestic animals, and wildlife, creating concerns about food safety, public health, and wildlife conservation.⁴⁹ Some of these diseases have existed for millennia, whereas others are emerging or reemerging, gaining the ability to jump between species and overloading traditional methods of disease surveillance and prevention. In a list of 1407 human pathogens, 58% are known to be zoonotic; 177 are categorized as emerging or reemerging, and zoonotic pathogens are twice as likely to be in this category as nonzoonotic pathogens.¹⁰⁰

The impact on human populations may be significant. The 2004 Joint United Nations (UN) Program on HIV/AIDS report on the global epidemic stated that mortality from human immunodeficiency virus (HIV) exceeded 20 million people in the 20 years since first diagnosed in 1980. HIV-1 and HIV-2 were introduced into humans through separate cross-species transmission of simian immunodeficiency virus. HIV-1 is believed to have arisen through transmission from chimpanzees and HIV-2 from sooty mangabeys (*Cercocebus atys*).⁴⁰

Wildlife species under severe environmental pressure are threatened by extinction from the spread of novel pathogens. Chytridiomycosis, caused by *Batrachochytrium dendrobatidis*, has been implicated in the massive mortality and global decline in a variety of amphibian species.⁴⁵ International trade is thought to play a key role in the worldwide dissemination of this disease.^{27,60}

Livestock production and market access to animal protein have been increasingly threatened by the emergence of disease. Since 1992, the economic damages from livestock diseases alone total more than \$60 billion. Outbreaks of bovine spongiform encephalopathy, foot-and-mouth disease, avian influenza, rinderpest,

and other diseases have prompted governments to impose trade embargoes and to mandate animal culling with increasing frequency. In 2003 the UN Food and Agricultural Organization (FAO) reported that one third of global meat trade was subject to embargoes because of disease outbreaks.²

The increase in infectious diseases may be linked to anthropogenic pressures of an urbanizing world, overall population growth, altered land use and agricultural practices, deforestation, global travel and commerce, microbial adaptation, and a weakened public health infrastructure. To forecast and respond proactively to the complex changes that influence the health of people, domestic animals, and wildlife, we must consider the driving forces that are affecting or will likely affect our world.

Globalization is the dominant international system that has made the world an increasingly integrated place, resulting in both threats and opportunities.³⁰ The global movement of people, animals, and their products has had profound effects on wildlife, livestock, and public health through the unchecked legal and illegal trade in exotic pets and bushmeat. *Human population increases* and the desire for improved standards of living promote *intensified agricultural practices*, pollution of air and water, as well as the unsustainable use of natural resources. There is little evidence to date that climate change has played a significant role in the resurgence of infectious disease. However, many believe that soon, *global climate change* will be responsible for regional climate alterations that affect physical and biologic systems.⁶⁸

These critical driving forces of globalization, human population increases with intensified agriculture, and global climate change provide a structure on which to consider exemplar emerging infectious diseases that imperil the future of humanity and animal life.



Fig 6-2 Cock fighting in a Thailand wet market. (See Color Plate 6-2.) (Courtesy RA Cook.)

Assuming an average body weight of 5 kg results in a conservative estimate of 200 million animals in central Africa and 12 to 35 million in the Amazon basin. The increasingly global scope of this trade, coupled with rapid modern transportation and the reality that markets serve as network nodes rather than as product endpoints, dramatically increases the movement and potential cross-species transmission of the infectious agents that every animal naturally hosts, as discussed next.

Monkeypox

Monkeypox is a rare, viral, smallpox-like disease from central and western Africa that was first diagnosed in laboratory primates in 1958. The first human cases were reported in 1970 in Africa. An outbreak in the Democratic Republic of Congo in 1997 was reported to have infected 88 people, with three deaths, all in children less than 3 years of age.³⁹

In late May and early June 2003, the first cases of a febrile rash illness in people were reported from Wisconsin, Illinois, and Indiana. Most affected people had been in close contact with recently purchased ill prairie dogs (*Cynomys*) that had been held with a recent shipment of African rodents. The African rodents that spread the disease had been legally shipped from Ghana to the United States (U.S.) in April 2003 for the pet trade. The shipment included a number of species, and studies indicated that two rope squirrels (*Funisciurus*), a Gambian rat (*Cricetomys*), and three dormice (*Dryomys*) were carrying the monkeypox



Fig 6-3 Pet howler monkey. (See Color Plate 6-3.) (Courtesy RA Cook.)

virus.³⁴ By early July, 71 nonfatal human cases from six states were reported to the Centers for Disease Control and Prevention (CDC).¹⁰ Before this event, nonendangered rodents from Africa were legally shipped into the U.S. for the pet trade with no regulatory controls. Subsequently, restrictions were placed on U.S. importation of African rodents.

Severe Acute Respiratory Syndrome

Severe acute respiratory syndrome (SARS) was first recognized as a newly emerging human disease in November 2002 in Guangdong Province, China.⁹³ Symptoms included high fever, respiratory illness progressing to pneumonia, in some cases diarrhea, and death. The disease first spread to Hong Kong and thereafter across five continents and 25 countries via infected people.⁷¹ In April 2003 a new coronavirus was discovered to be the causative agent. In July 2003 the World Health Organization (WHO) listed the number of probable SARS cases in humans at 8437, with 813 deaths.⁸ Evidence of viral infection, often without signs, was also detected in palm civets (*Paguma*) farmed in the region.³³ The initial suggestion of a link between civets and SARS led to a government directive to cull more than 10,000 masked palm civets in the province despite the ambiguity of the disease link.⁹ Later, viral evidence was also detected in raccoon dogs (*Nyctereutes*) and ferret badgers (*Melogale*) as well as domestic cats. It now appears that the palm civet served as an artificial market-induced host or amplification host, along with a number of other possible species. Subsequent

studies determined that three species of horseshoe bat (*Rhinolophus*)²⁸ were found to be the natural reservoir host for closely related SARS-like coronaviruses.^{51,56}

Bats have been found to be reservoir hosts for a number of viral pathogens, including Lyssa, Nipha,¹⁰¹ Hedra, and Ebola viruses. Their role in emerging disease spread appears to be significant.

Ebola

Ebola hemorrhagic fever (Ebola) is named after the river in the Democratic Republic of Congo (DRC, formerly Zaire), where it was first identified. Chimpanzees and humans share 98% of their DNA, and gorillas and humans share 97%.⁸⁰ Therefore, bushmeat in the form of nonhuman primates poses a particularly high risk of cross-species infection into humans. The first three known outbreaks of Ebola occurred between 1976 and 1979 in DRC and Sudan. Between 2000 and 2004, five human Ebola outbreaks were documented in western-central Africa. Epidemiologic studies indicated that these outbreaks resulted from multiple introductions of virus from infected animal sources. The index cases were mainly hunters, and all were infected while handling dead animals, including gorilla (*Gorilla*), chimpanzee (*Pan troglodytes*), and duiker (*Cephalophus*).⁵³ Thereafter, outbreaks spread quickly between people, especially through caregivers, and were documented to almost wipe out entire villages.^{31,52} In people the symptoms are referable to multiple organ effects with internal and external hemorrhaging. The Zaire subtype of Ebola virus has been known to have a case-fatality rate of almost 90%, and the Sudan subtype has a rate of approximately 50%.⁸²

Ebola has been linked to declines in western equatorial Africa great ape populations. There is evidence that other forest animals, such as the duiker, are also affected.⁵³ Data do not exist on total numbers of nonhuman primates and duikers that have died of the disease, but it is believed that Ebola rivals hunting as the major threat to ape populations.⁹² For some time the natural reservoir host remained elusive.⁷⁵ Bats were long postulated as a potential reservoir host, as recently confirmed in three species of fruit bat.⁵⁴

The movement of nonhuman primates for use in biomedical research has also proved to be a source for the spread of Ebola-related viruses. In 1989, a closely related simian hemorrhagic fever was diagnosed in Reston, Virginia, in imported cynomolgus monkeys from the Philippines that died during quarantine. Named Ebola Reston, the disease was later found not to cause human disease.⁶²

EFFECT OF HUMAN POPULATION GROWTH ON AGRICULTURAL PRACTICES

By July 2005, the world had an estimated 6.5 billion human inhabitants, 380 million more than in 2000. About 95% of all population growth is occurring in the developing world and 5% in the developed world. By 2050, it is estimated that the world population will increase by 2.6 billion.⁶ For the 50 years preceding 2000, agriculture focused on meeting the food, feed, and fiber needs of a growing human population. In the next 50 years, the challenge will be not only feeding an expanding human population, but also doing so in a world of declining resources, including water and arable land.⁴⁷

Large-scale agriculture is susceptible to outbreaks of disease. The 1983–1984 poultry epidemic of highly pathogenic avian influenza in the Northeast U.S. caused markets to drop by \$349 million during the 6-month period of the disease.¹⁸ The economic impacts of the Nipah virus outbreak in Malaysia in 1997–1998 was estimated to cost \$350 to \$400 million, whereas the 2001 foot-and-mouth disease outbreak in England and Europe was estimated to have cost markets almost \$30 billion (U.S. dollars).⁶⁶ In the developed world, agribusiness and government commitments to quality farm practices and rigorous health inspection have created a predominantly safe food supply. To provide food animal protein at the levels required, the industry has moved toward more intensive practices that increase productivity through selective breeding for desirable market traits and large-scale biosecure facilities. These characteristics may also leave operations vulnerable to the introduction and rapid spread of pathogens via errant contact with wildlife or the global movement of animals and products from areas that do not practice similar levels of biosecurity.

Developing-country livestock practices are highly different. Often, livestock share space with people in and around the home. The rearing of ducks in Asia is an efficient system in which domestic ducks and geese are given access to recently harvested rice paddies. This allows wild waterfowl and domestic species to mix, however, creating an environment conducive to the cross-species spread of pathogens.

Transmissible Spongiform Encephalopathies

The transmissible spongiform encephalopathies include chronic wasting disease of cervids, *scrapie* of sheep,

bovine spongiform encephalopathy (BSE) of cattle, and *Creutzfeldt-Jakob disease* (CJD) of people. They are caused by pathogenic *prions*, which are transmissible particles devoid of a nucleic acid genome and composed of a modified isoform of normal prion protein.⁷⁷ These prion proteins are extremely resistant to inactivation by ultraviolet light, ionizing radiation, steam sterilization, and almost all forms of traditional disinfection.

High-volume food production needs prompted the livestock industry to begin feeding ruminant protein to cattle, possibly derived from scrapie-infected sheep. It is believed that this practice led to the outbreak of BSE in the United Kingdom (U.K.), which then spread to continental Europe, Canada, and more recently the U.S. It was likely through the ingestion of prion-infected meat from cattle that a new emerging disease of people was discovered in 1996, *variant Creutzfeldt-Jakob disease* (vCJD).

From October 1996 to November 2002, 129 cases of vCJD were reported in the U.K., six in France, and one each in Canada, Ireland, Italy, and the U.S.¹¹ The World Organization for Animal Health (OIE) listed more than 184,296 cases of BSE in U.K. cattle alone as of September 2005. As confirmed, 13 species of zoo animals, including bovidae and felidae, have died as a result of infection with the BSE agent.²⁵

Chronic wasting disease (CWD) is a prion disease of wild and farmed cervids in North America.⁹⁷ It was first recognized in a research herd of mule deer (*Odocoileus hemionus*) in Colorado in 1967. In 1985 it was diagnosed first in elk (*Cervus elaphus*) and then in mule deer in a limited region of Colorado. It is believed that the increase in deer and elk farming and the movement of animals for that industry in the U.S. and Canada provided a means for spread. It has since been diagnosed in multiple states and regions both in captive and free-ranging cervids. Conversion of human prion protein by CWD-associated prions has been demonstrated in an in vitro cell-free experiment,¹⁵ but to date, investigations have not identified evidence for CWD transmission to humans.¹⁴

Avian Influenza

Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus. Wild birds, predominantly ducks, geese, and shorebirds, are the reservoir species for the *low-pathogenic* strains of *avian influenza* A virus (LPAI) in nature.⁹⁵ In these species it does not usually cause illness. The virus is subtyped on the basis of the antigenic properties of hemagglutinin (HA, or H) and neuraminidase (NA, or N) glyco-

proteins; 16 HA and 9 NA subtypes have been demonstrated. Viruses containing subtypes H5 and H7 have been observed to become *highly pathogenic avian influenza* (HPAI) in poultry. HPAI has been isolated primarily from commercially raised birds, including chickens, turkeys, quail, guinea fowl, and ostrich (*Struthio camelus*). Influenza A viruses of the H5 and H7 subtypes have also been detected in a variety of mammals, including humans. The H5N1 influenza A viruses have been detected in birds, pigs, cats, leopards, tigers,⁴⁴ and people in Asia.⁶⁴

Live-bird markets that sell a wide variety of domestic and wild bird species to the public provide the perfect conditions for genetic mixing and spread of flu viruses.⁹⁴ In addition, traditional poultry livestock practices that bring people into close contact with domestic fowl and promote the mixing of wild and domestic waterfowl also provide opportunities for domestic-wildlife viral exchange and spread into humans. Such an occurrence may have been the cause of the avian flu (H5N1) outbreak in Hong Kong in 1997 and again in late 2003–2004 throughout Asia. Once established in poultry in Asia, a combination of intensive production methods and high-volume poultry movement in addition to poor sanitation and hygiene allowed the disease to spread.

In 2005 the H5N1 HPAI was isolated from migratory waterfowl on Quinghai Lake, China,²¹ and from a wild whooper swan in Mongolia.¹ However, it remains unclear whether migratory waterfowl are effective carriers of the disease or rapidly succumb to the infection before they spread the disease, as may have happened in Mongolia. Calls for mass culling of wild birds have been countered by conservation groups and the FAO.⁴

Of greater concern should be the global trade in domestic and wild birds. An illegal shipment of two crested hawk-eagles (*Spizaetus nipalensis*), smuggled into Europe from Thailand, was seized at the Brussels International Airport in October 2004. Both birds appeared clinically normal, and both were positive for the H5N1 HPAI.⁹¹

The threat posed by avian influenza goes beyond the food supply to becoming a lethal virus that is easily spread between people, a *global pandemic*. Such a scenario portends grave risk to the economies of nations and to the health of people. The report of the U.S. National Intelligence Council identified a global pandemic as the single most important threat to the global economy.³⁸ As of December 2005 the WHO confirmed 142 human cases, with 74 resulting in death. These tragic statistics pale compared with the greater human disease threat. Genetic reassortment

of the H5N1 precursor viruses that caused the initial human outbreak in Hong Kong in 1997 may be traced to outbreaks in poultry in China and seven other East Asian countries between 2003 and early 2004. This same virus has been fatal to humans in the region.⁵⁵ The fear is that the H5N1 viruses will gain the ability to spread efficiently among people, causing a global pandemic.

There is good reason for concern: in the twentieth century there have been three global pandemics, all believed to have originated from birds.⁷³ The most severe was the 1918 Spanish influenza pandemic virus (H1N1), which was estimated to have killed 20 to 50 million people worldwide. Pandemic influenza may originate through at least two mechanisms: (1) reassortment between an animal virus and a human virus that yields a new virus and (2) direct spread and adaptation of a virus from animals to humans.¹⁶ The characterization of the reconstructed 1918 Spanish influenza pandemic virus⁸⁴ showed that the direct spread and adaptation of the avian influenza virus caused the pandemic.

GLOBAL CLIMATE CHANGE

Projections using emissions scenarios based on a range of climate models suggest an increase in global average surface temperature of 1.4° to 5.8° C over the period of 1990 to 2100. This projected rate of warming would be unprecedented based on at least the last 10,000 years.⁶⁸ The health of people and animals may be impacted by an increase in the frequency and severity of climate extremes (storms, floods, heat waves, etc.) and climate-induced changes in the geographic distribution and biologic behavior of arthropod vector-borne²⁶ and rodent-borne infectious disease.⁶¹ Climatic factors such as increased temperature, increased or decreased precipitation, and sea-level rise may all have an impact on the emergence or reemergence of infectious diseases.

Climate plays a critical role in the maintenance of vector species as well as pathogens. Studies suggest that warming will enhance transmission intensity and extend the distribution of certain vector-borne diseases.^{69,78} The complexity of ecologic systems and human-induced changes to the environment make it difficult to establish definitive links between predicted climate-induced changes and emerging arthropod vector-borne and rodent-borne diseases. However, there are a number of diseases to consider as candidates.

ARTHROPOD VECTOR-BORNE DISEASES

Vector organisms, such as mosquitoes and ticks, transport pathogens from an infected individual or its wastes to susceptible individuals, their food, or immediate surroundings. Climate alterations may affect the distribution of vector species, changing their range because of altered conditions for breeding and feeding. Temperature may also impact survival rates of both the pathogen and the vector organism, further influencing disease transmission. The range of the major arthropod vector-borne zoonotic pathogens includes both parasitic and viral diseases. Parasitic organisms spread by vectors include malaria (*Plasmodium*), Chagas' disease (*Trypanosoma cruzi*), Lyme disease (*Borrelia burgdorferi*), and leishmaniasis (*Leishmania*). The vector-spread arboviruses include organisms in the family Flaviviridae (e.g., St. Louis encephalitis, dengue fever, yellow fever, West Nile virus), Bunyaviridae (e.g., La Crosse virus), and Togaviridae (e.g., eastern, western, and Venezuelan equine encephalitis).

Lyme Disease

Lyme disease is transmitted primarily by the deer tick *Ixodes scapularis*. It is the most common vector-borne disease of people in the U.S.³⁶ and is perpetuated in a life cycle that involves rodent reservoir hosts, such as white-footed mice (*Peromyscus leucopus*) in eastern North America and *Apodemus* mice in Eurasia. Lyme disease spirochetes have been shown to infect a diverse number of mammals and birds, but not all have been shown to serve as competent hosts.⁷⁹ Symptoms in humans may include erythema migrans in approximately 50% of patients, and signs in both humans and other mammals may include fever, lameness, listlessness, anorexia, lymphadenopathy, and joint swelling. Other signs referable to affected systems may include reproductive difficulties and arthritides.

The *dilution effect* model suggests that the loss of the diversity of vertebrate reservoir hosts caused by anthropogenic forces may increase the spread of Lyme disease. As habitats are degraded, the diversity of potential hosts decreases. Many members of this diverse group of potential tick hosts are less competent as Lyme disease reservoirs. These species-poor communities tend to have low levels of those species that are less competent as reservoir hosts and high levels of the most competent disease reservoir, the white-footed mouse (*P. leucopus*).⁵⁸

The influence of climate change on the distribution of *I. scapularis* and the spread of Lyme disease in North America has been modeled.¹⁹ Projections suggest that the range of the tick will extend into Canada. In addition, as a disease primarily carried by rodents, climate change may further impact the distribution of Lyme disease by altering the range of the rodent hosts.

West Nile Virus

West Nile virus (WNV) was first isolated from the blood of a febrile woman in the West Nile district of Uganda in 1937.⁸³ Thereafter it was isolated from ill people, birds, and mosquitoes in Egypt during the early 1950s. WNV is recognized as the most widespread of the flaviviruses, with geographic distribution in Africa and Eurasia.³⁷ The disease entered the Western Hemisphere in New York in 1999, with deaths observed in humans, horses, and many species of wild birds, though primarily corvids. The virus subsequently has spread across North America⁷⁴ and into tropical America and the Caribbean.^{50,48}

WNV presentation varies with the species and may range from no signs to death, with the typical illness including encephalitis and fever. The CDC Division of Vector Borne Disease notes that more than 284 species of birds as well as both domestic and wild mammals have been affected.

Although bird-feeding species of mosquito are the principal vectors, WNV has been isolated from numerous additional species of mosquito as well as from ticks.³⁷ Global climate change alterations that include warmer temperatures with higher humidity would favor the increase in abundance and distribution of the mosquito vectors.⁷⁸

Dengue Fever

Dengue fever (DF) is caused by one of four closely related but antigenically distinct virus serotypes of the genus *Flavivirus*.³⁵ It is the most common vector-borne disease of humans, infecting an estimated 50 million people in tropical and subtropical regions of the world each year.⁸⁹ In humans, symptoms of DF may range from inapparent to a mild, influenza-like illness to an immune-mediated hemorrhagic fever that may be fatal if untreated.⁸⁵

Dengue viruses are transmitted between people or between monkeys through mosquitoes of the genus *Aedes*. Estimates are that the zoonotic transfer of

dengue from monkeys to sustained human transmission occurred between 125 and 320 years ago.⁸⁹ DF is endemic in approximately 100 countries in Southeast Asia, Africa, the western Pacific, the Americas, Africa, and the eastern Mediterranean.²⁰

The reasons for the global emergence of DF are multifactorial and include ineffective mosquito control, major urbanization shifts in demographics, and unimpeded international travel, which allows people to move the virus into new population centers. However, climate change is also implicated as a potential future factor; modeling studies project that a warming of 2° C by 2100 will result in a net increase in the potential latitudinal and altitudinal range of DF and an increase in duration of the transmission season in temperate locations.⁶¹

RODENT-BORNE DISEASES

Rodent-borne diseases spread from species to species through contact with rodent urine, feces, or other body fluids. Climate change factors that may expand the range and increase the reproductive potential of rodent populations include increased rainfall, warmer temperatures, and climatic extremes.

Leptospirosis

Leptospirosis is a reemerging zoonotic disease of global importance that occurs in urban settings of both industrialized and developing countries¹⁷ as well as in rural environments worldwide. It affects domestic livestock,⁴³ alternative livestock,⁵⁹ as well as both free-ranging⁹⁰ and captive⁵⁷ wild mammals, including marine mammals.²³ Both rodents and dogs are important vectors⁶³ in the urban and agricultural setting. The transmission of leptospirosis occurs most often through contact with water contaminated by urine from infected shedders.

Leptospirosis is caused by a filamentous spiral bacterium that has a predilection for renal tubules. In people the disease symptoms may range from subclinical to epidemic leptospirosis, associated with pulmonary hemorrhage, renal failure, and jaundice. In domestic animals as well as captive and free-ranging wildlife, the animals may appear clinically normal or may present with disease signs referable to renal and reproductive tract infection. In captive wildlife, leptospirosis is often an insidious infection that may result in chronic renal disease and high rates of reproductive failure.

Rodent populations in temperate and tropical climates may serve as the reservoir host for domestic and wild animals as well as human infection. Outbreaks of human disease are often associated with increases in rodent populations after heavy rainfall or during floods.^{88,65}

Hantavirus

Hantaviruses (genus *Hantavirus*) cause two major clinical syndromes of people in different areas of the world. Hemorrhagic fever with renal syndrome is seen in Asia and Europe, whereas a pulmonary syndrome is described in the Americas.⁷⁶ Hantavirus is a zoonotic virus of rodents and has emerged as a human pathogen as a result of human-induced landscape alterations and climatic changes influencing population dynamics of the rodent reservoir hosts.⁹⁸

Outbreaks of disease may be associated with weather that promotes rapid increases in the rodent population, which may vary seasonally and annually.³² A study of the relationship between climate and the prevalence of *Hantavirus* pulmonary syndrome in Arizona, New Mexico, Colorado, and Utah region found an increase in rodents was associated with increased precipitation patterns during the 1992–1993 El Niño. An outbreak of *Hantavirus* pulmonary syndrome occurred in this region during 1993.²⁹

INTEGRATED APPROACH TO WORLD HEALTH: ONE WORLD, ONE HEALTH

The dramatic increase in emerging disease outbreaks and the growing number of diseases moving between species indicate that traditional approaches to disease management, currently segregated into human, livestock, and wildlife health, are not effective. In addition to the suffering and death, these diseases result in billions of dollars spent on reacting to outbreaks that would be more efficiently spent on prevention. We must seek to be *proactive*, to prevent rather than react, and more thoughtfully control global movement of people and animals. To devise sound solutions, we must develop a unified approach that incorporates the knowledge and experience of broad areas of science and health. This approach must be *flexible* to respond to novel threats, *adaptive* to react to changing situations, *distributive* to monitor change at the global scale, and *inclusive* to learn from the varied experience of human, domestic animal, and livestock disease knowledge.²⁴ In short, a “one health” approach is needed.^{41,5,67}

Although this concept is centuries old, the application is new: there is only “one health” for people, domestic animals, and wildlife.

Changing the Global Perspective

Society has not yet fully adapted to the changes brought on by globalization. Multilateral organizations are responsible for public health (WHO) and livestock health (FAO, OIE), but there is no similar umbrella organization to bring together the worldwide focus on wildlife health. Until such a body is formed, wildlife will continue to be a footnote to human and domestic animal health perspectives and will continue to be the surprise critical variable in emerging disease spread.

The World Trade Organization (WTO) and other appropriate international bodies must start requiring governments to better regulate the health aspects of international trade in wild and domestic animals. Individual nations must implement and enforce laws to prevent the spread of diseases within their borders. It is clear that trade and the consumption of wildlife have led to global health disasters; governments must therefore make greater efforts to reduce and properly regulate the wildlife trade internationally, regionally, and locally.

Role of Zoo and Wildlife Health Professionals

The complexity of the emerging disease and health issues that humanity confronts at the interface of people, domestic animals, and wildlife requires a multidisciplinary approach to problem solving. Zoo and wildlife health professionals have the unique, comparative systems perspective that is essential to the multidisciplinary team. Surveillance for the emergence of new diseases is a critical component of a sound approach to disease mitigation, control, and prevention. For example, the veterinary health professionals from zoos of the Association of Zoos and Aquariums (AZA) participate in reporting systems for tuberculosis monitoring in hoofstock⁸⁶ and a national surveillance system for WNV in zoologic institutions.⁸⁷ This is a significant first step, but it must be followed by expanded efforts for broader disease surveillance on a global scale.

To be effective, the profession must establish the collaborative and communications links with public health and domestic animal health agencies, diag-

nostic centers, human and veterinary medical facilities, and university-based health research institutions. New public-private partnerships must be fostered with the corporate sector. The multinational industries best understand the threat to global supply chains, economies, and the health of both their employees and the consuming public. Overall, the zoo and wildlife health professionals must become active participants and advocates for a broader view of health.

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Color Plate 6-1 South African market with bushmeat for sale. (For text mention, see Chapter 6, p. 56.) (Courtesy RA Cook.)



Color Plate 6-2 Cock fighting in a Thailand wet market. (For text mention, see Chapter 6, p. 57.) (Courtesy RA Cook.)



Color Plate 6-3 Pet howler monkey. (For text mention, see Chapter 6, p. 57.) (Courtesy RA Cook.)

CHAPTER 7

Behavioral Training for Medical Procedures

TIMOTHY A. REICHARD

Training animals to cooperate voluntarily in veterinary procedures is an important cornerstone of a zoo's animal care program and provides numerous benefits.⁷ Preventive medicine tasks, measurement of baseline physiologic parameters, visual and physical examinations, diagnostic procedures, therapy, and reproductive evaluations may be done more efficiently with less stress to the animals and without the inherent risks of anesthesia. Long-term therapy such as insulin injections, impossible in many cases in the past, may frequently be accomplished.⁹ Animal introductions may occur with less stress and fewer injuries.⁴ The training process may desensitize the animal to past negative experiences with the veterinarian, thus developing trust between animal and caregiver and allowing closer observation of the animal. Often overlooked are the positive psychologic stimulation and behavioral enrichment benefits that the hours of training also provide for the animal.

FINANCIAL BENEFITS OF A TRAINING PROGRAM

Along with the benefits of a training program come costs. There may be a substantial commitment of time by both the keeper/trainer and the veterinary staff. The risks of staff injuries increase when fully aware animals are palpated and body parts manipulated. Limbs of staff may be grabbed, bitten, and crushed with subsequent lacerations and fractures. Expensive medical equipment may also be at risk of damage by animals.

Benefits of training for medical procedures are maximized and costs minimized when a carefully planned program is developed and implemented. Important components include the following:

1. The designation of a program coordinator, assignment of roles and responsibilities for all involved (curators, keepers, veterinary staff), and a com-

munication flowchart. The program coordinator should be an animal behavior manager, a keeper experienced in training, or a consultant animal trainer.

2. Identification, selection, and prioritization of medical behavioral projects.
3. Training of keepers and veterinary staff in the theory, terminology, and application of positive-reinforcement operant-conditioning techniques.⁶
4. Assistance and support of the keeper/trainer and veterinary staff during the animal-training process.
5. Ongoing assessment and evaluation of program progress and results.

A well-informed, skilled staff increases training effectiveness and the efficiency and reliability of positive results.⁸ Skillful application of techniques reduces risk of injury and avoids excessive frustration for trainers and the animals. A helpful tool is a medical behavior training worksheet that includes a brief description of the behavior, name of trainer, training steps, equipment needs, and safety concerns and mitigation steps. The worksheet is prepared and reviewed with input by program coordinator, curators, trainers, and veterinary staff, and progress is reviewed at designated times during the training process.

HUSBANDRY-RELATED BEHAVIORS

The following simple, but valuable, husbandry-related behaviors are important for early training. Operant conditioning for efficient and timely movement of animals between holding areas and exhibit spaces is important for cleaning and sanitation, distribution of enrichment items such as food treats, and isolation of ill or injured animals. The ability to isolate an animal from the group is required for visual and physical examination, weight monitoring, food restriction, immobilization, and administration of medications. Training an animal to step onto or sit on a scale for

weighing aids in body condition evaluations, weight management, and determination of proper drug dosages for immobilization and medication. Unnecessary immobilizations may be necessary if an animal cannot be isolated for evaluation of the severity of bite wounds. In one institution it was impossible to separate individuals in the common chimpanzee (*Pan troglodytes*) troop, and three animals had to be immobilized at once, substantially increasing the anesthesia risks. Escaped animals that have been previously trained may sometimes be coaxed back into a holding area by their keeper without immobilization.³

The training of behaviors such as blood collection, hand injection, and palpation of body parts that involves direct physical contact between the animal and the trainer and veterinary staff requires safety precautions. The trainer should not work alone and needs ready access to a portable radio, and the animal should be properly confined. Training an animal to enter and remain calm in a confined space, such as a small holding area, squeeze cage, or crate, provides better access, restricts movements of the animal, and reduces the risks of staff injuries.⁴ Often the animal needs no further restraint. Once movement is limited, tails or limbs may be more safely accessed for blood collection, blood pressure measurement, and injection. Anesthetic agents may be injected with greater accuracy and with less animal excitement, resulting in smoother, faster, and safer inductions.

The following examples of behaviors have been trained in marine mammals, primates, hoofstock, and carnivores. Being able to perform these medical procedures without anesthesia is particularly valuable for species such as the reticulated giraffe (*Giraffa camelopardalis*) and Nile hippopotamus (*Hippopotamus amphibius*) that are difficult and risky to immobilize even when healthy.² Detailed descriptions of the training process for several of these behaviors may be found in the references. Also, experienced colleagues are ready and willing to assist.

Recording baseline physiologic parameters is valuable for assessing health concerns. Animals have been trained for measurement of body temperature, heart rate, and blood pressure; blood collection for hematologic values and serum biochemical constituents; and urine collection for urinalysis and hormonal assay.¹⁰ In the past these measurements could be taken in many species only under anesthesia, which in itself can induce changes in the parameters.

Examination of ill animals by palpation of limbs to detect pain, rectal palpation, checking severity of wounds, observation of oral pathology, and auscultation of the heart and lung may be accomplished.

Diagnostic procedures such as radiology, ultrasonography, biopsies, cultures for pathogens, and tuberculin testing have been performed without the risk of anesthesia, which is especially valuable in sick animals.¹ Treatments that have been administered without anesthesia or physical restraint include daily injections of medications, minor surgical procedures, wound care, and bandage changes. Training of hoofstock to stand or lie down for periodic foot and hoof care is invaluable for the successful resolution of foot pathology.⁵ Animals also have been trained to cooperate in reproduction-related procedures, including semen collection, artificial insemination, collection of milk, and bottle feeding of babies being cared for by surrogate mothers.⁸

The list of trained behaviors in zoo animals is almost endless. With patience, adequate trainer skill, staff cooperation, and creativity, almost any behavior may be trained.

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CHAPTER 8

“Balai” Directive of the European Union: Difficult Veterinary Legislation

PETER DOLLINGER

THE ORIGINAL “BALAI” DIRECTIVE

On 13 July 1992, the Council of the European Communities adopted the “Directive 92/65 laying down the animal health requirements governing trade in and imports into the Community of animals, semen, ova and embryos not subject to animal health requirements laid down in specific Community rules referred to in Annex A(I) to Directive 90/425/EEC.”¹ This directive is more concisely referred to as one of two “Balai” directives, the other being Directive 92/118/EEC, governing trade in certain products under animal and public health criteria. The term *balai* is French, meaning “broom.” It is used in this context because, with a view of completing the European Union’s internal market, all veterinary issues that were not yet regulated were swept together and packed in the two directives. Because of time constraints, Directive 92/65 was prepared hastily and without consulting with the zoo community. It was poorly drafted, in particular its English version, and thus unclear, misleading, and impractical. Even the veterinary services of the member states apparently were taken by surprise when the European Union (EU) Council signed the proposal as a directive in July 1992, to take effect on 1 January 1994.

Neither the authorities of the member states nor the commission itself seemed to appreciate the different animal species involved or the type and number of annual movements of animals between zoos, or that the majority of these transactions were for conservation breeding purposes. In practical terms, the directive proved to be an obstacle for the exchange of zoo animals rather than facilitating these movements. Consequently, zoos hesitated to seek approval under the directive, and national governments were rather slow in implementing it.

INVOLVEMENT OF VETERINARIANS IN REVISION OF DIRECTIVE

After the European Association of Zoo and Wildlife Veterinarians (EAZWV) was founded in 1996, it became a driving force for revision of the “Balai” Directive, with considerable effort to communicate with EU officials. Expert work was concluded in March 2001, and the amended directive adopted by the EU Council on 15 July 2002.

Under the prevailing legislative procedures in the EU, amending the core body of the directive was difficult. Instead, Annex A, containing the list of diseases relevant for the directive; Annex C, defining the conditions for approval; and Annex E, containing model certificates, were amended. Because the amendment was done in the legal form of a regulation, annexes A, C, and E became directly applicable in member states, which should guarantee a relatively uniform application. EAZWV wanted the implementation as uniform as possible, because only this would ensure that the sanitary level of all approved zoos followed the same high standard, which would permit the circulation of animals between approved zoos with minimal health risks.

This implied that zoo veterinarians (1) were fully aware of the provisions of the revised “Balai” Directive, (2) would take their obligations under the directive seriously, (3) would approach their duties in a uniform way, and (4) were also prepared to cover a number of diseases not, or not explicitly, addressed by the directive. To this end, EAZWV organized a “Balai” workshop at its 2002 conference in Heidelberg, presenting the contents of the revised “Balai” Directive to zoo veterinarians and directors at different meetings. EAZWV also established an infectious diseases working group, chaired by Jacques Kaandorp of

Beekse Bergen Safari, with the mandate of developing a transmissible diseases handbook,⁶ and hired a part-time veterinarian to act as secretary of the working group.

During the negotiations, two surveys were conducted to ascertain how EU member zoos were using the original “Balai” Directive. Few substantial answers were received, and it became clear that veterinary services had no uniform interpretation of the directive; most had not even seriously dealt with the new piece of legislation. Compliance was highly variable. Most respondents wanted a revision, although a few were satisfied with their approach. It was evident that quarantine facilities and record keeping were inadequate.

DEVELOPMENT OF RECOMMENDATIONS FOR APPLYING THE DIRECTIVE

The conclusion from the two surveys was that EAZWV should provide guidance to both zoos and veterinary services throughout the EU as well as in Andorra, the British Crown Dependencies (Channel Islands, Isle of Man), Liechtenstein, Monaco, Norway, San Marino, and Switzerland, where the “Balai” Directive also is applicable, either under the Agreement on the European Economic Area (EEA) or under bilateral treaties.

To this end, two meetings were held on 15/16 September 2003 and 5 February 2004 at Cologne Zoo with the participation of representatives of the European Commission (DG SANCO: Health and Consumer Protection); British Department for Environment, Food & Rural Affairs (DEFRA); Dutch Rijksdienst voor de Keuring van Vee en Vlees (RVV); German Bundesministerium für Verbraucherschutz, Ernährung und Landwirtschaft (BMVEL); the EAZWV, in particular its Infectious Diseases Working Group (IDWG); and zoo veterinarians from France, Germany, Italy, The Netherlands, and the United Kingdom, representing also their respective professional organizations at the national level.

The recommendations, which were finalized on 20 February 2004, were disseminated as part of the EAZWV *Transmissible Diseases Handbook*⁶ by EAZWV and the European Association of Zoos and Aquaria (EAZA) to their respective constituencies and by the EU Commission to the veterinary services of the member states, which implied that they received some type of official status. The recommendations contain six chapters dealing with the term “animals,” the

approved veterinarian, annual disease surveillance plan, added-animals procedure, quarantine/isolation requirements, and the certificates:

COMPONENTS OF THE REVISED “BALAI” DIRECTIVE

The Term “Animals”

“Animals” in the terms of Article 2(b) of the directive means “specimens of animal species other than those referred to in Directives 64/432/EEC, 90/426/EEC, 90/539/EEC, 91/67/EEC, 91/68/EEC, 91/492/EEC and 91/493.” The average zoo veterinarian has no clue what this means, and even many official veterinarians may have problems finding out what exactly is covered by the directive, and what is not. Therefore a list of the species covered by the various other directives is provided. The recommendations state, however, that it would make no sense to exclude these species not addressed by the “Balai” Directive from the health surveillance plan.

Approved Veterinarian

To be granted official approval under Article 13 of the directive, a zoo must secure by contract or legal instrument the services of a veterinarian approved by and under the control of the competent authority. The role of the approved veterinarian is to ensure that the requirements of the “Balai” Directive and other related legislation are complied with on a day-to-day basis. Where this veterinarian is a member of a practice, other members of the same practice may be included, provided that they are also approved by the competent authority and individually nominated in writing.

Also in the case of the approved veterinarian, the “Balai” Directive refers to other EU legislation by requiring that approved veterinarians comply *mutatis mutandis* with the requirements referred to in Article 14(3)(B) of Directive 64/432/EEC; the recommendations explain what this exactly means. It was agreed with the EU Commission that one of these requirements, according to which the approved veterinarian must have no financial interest or family links with the owner of or person responsible for the holding, could be interpreted liberally because zoo animals have a conservation value rather than an economic value and because, for the purposes of the “Balai,” the approved veterinarian is working under the supervision of the

official veterinarian. It is thus the official veterinarian's duty to assess whether a conflict of interest could exist, and whether the veterinarian appointed by the zoo fulfills the requirements for being approved, and in particular has appropriate specialist knowledge in relation to zoo animals.

Annual Disease Surveillance Plan

The approved veterinarian must draw up and implement an annual disease surveillance plan. This plan is subject to annual audits by an official veterinarian from the competent authority. The recommendations explain that, for the purposes of approval under the "Balai" Directive, the surveillance plan must cover those diseases listed in Annex A (and B if relevant), and suggest that the plan may also include other general measures as may be required under Council Directive 1999/22/EC of 29 March 1999, relating to the keeping of wild animals in zoos,² and specific measures for individual taxonomic groups as may be agreed by the relevant Taxonomic Advisory Group of the European Endangered Species Program (EEP) of the EAZA. As a general rule, such specific measures would be elaborated by the EAZWV IDWG and subsequently integrated into the Husbandry Guidelines for the taxon concerned.

As agreed with the representatives of the EU Commission and the national veterinary services participating in the Cologne meetings, the annual disease surveillance plan and associated measures must include the following:

1. Immediate notification to the competent authority if there is any suspicion that animals may be affected by any disease, including zoonoses, that is notifiable under EU legislation or national legislation.
2. Close observation of each animal at least once per day by suitably qualified staff, under the direction of the approved veterinarian. In the case of large group species, such as fish in an aquarium, the veterinarian may decide that observation of the group is sufficient.
3. Immediate notification of the approved veterinarian by zoo staff if any animal appears to be sick or dies. In the case of large group species, notification may be triggered by mortality above an agreed, expected level.
4. Laboratory examination to establish the infective agent in any live animals that appear to have an infectious disease. In the case of large group

species the veterinarian may decide that a representative sample is sufficient. In the case of a suspected disease listed on annexes A and B or notifiable under national legislation, the official veterinarian must be informed immediately.

5. Procedures for newly arrived and diseased animals, taking into account the relevant risk factors.
6. Regular parasitologic examination of fecal samples, especially for zoonotic parasites. All relevant groups should be checked at least annually; the frequency of examination should be related to the prevalence of parasites.
7. Opportunistic examination of and appropriate samples from immobilized or otherwise-restrained animals. All serum samples should be retained and stored at -18°C or lower.
8. Guidelines for the systematic testing of specific animal species may be developed and recommended by the IDWG of EAZWV.
9. Postmortem examination without unnecessary delay to check for significant pathology and, as possible, to establish the cause of death in every animal or aborted fetus unless there is clearly no suspicion of infectious disease.
10. The vaccination program should be based on the availability of safe vaccines. It should take into account the species involved and the risk of diseases likely to occur in the zoo and may cover zoonotic diseases other than those mentioned in Annex A or B, but these vaccinations must be in compliance with the applicable legislation.
11. Records must be kept in an easily accessible form, to be available as necessary for audit purposes, and retained for at least 10 years. The recommendations define in detail which information the records must contain.

Added-Animals Procedure

The fact that no animals originating from nonapproved sources (unless imported from "Third Countries") could be added to an approved collection was the main obstacle that prevented zoos from seeking approval under the original "Balai" Directive. The introduction of an "added-animals procedure" was thus the main prerogative for normalizing the situation. Under the revised "Balai," animals from nonapproved sources may be introduced to an approved collection provided that certain conditions are respected. The recommendations examine the various situations and provide guidance on how to handle them:

Animals coming from another approved establishment in the same member state fall outside the scope of Directive 92/65, and thus under EU legislation there is no requirement for the animal to be accompanied by the model health certificate in Annex E. However, national rules governing certification may apply. For the same reason, there is no official requirement for postarrival isolation, although the establishment may choose to carry out isolation and testing for its own private purposes. If the animals are coming from an approved establishment in another member state, they must be accompanied by the relevant model health certificate in Annex E. Depending on the health situation, additional requirements may be imposed by EU or national legislation.

Animals coming from a nonapproved establishment in the same member state fall outside the scope of Directive 92/65, and thus again, under EU legislation there is no requirement for the animal to be accompanied by the model health certificate in Annex E. In accordance with Annex C of Directive 92/65, however, the animals must undergo postarrival isolation in the isolation area, designated in the terms of approval, for at least 30 days or such longer period as may be required by the approved veterinarian and/or the competent authority to be satisfied that the health status of the animals is not inferior to the health status of the other animals in the collection. During isolation the animals may be required to undergo testing for any disease covered by Annex A of the “Balai” Directive that the approved veterinarian and the competent authority consider appropriate.

Member states may, by way of exemption, allow the movement of animals from nonapproved establishments in another member state. Specific conditions under which transfer must occur may be laid down. The animals must undergo postarrival isolation in the isolation area, designated in the terms of approval, for at least 30 days or such longer period as may be required by the approved veterinarian and/or the competent authority.

In the situation of animals from a nonapproved establishment to a nonapproved establishment within the same member state, national rules apply. Between establishments in different member states, the member state of destination may request specific requirements for introduction.

Animals being imported into the community from Third Countries must fulfill the animal health conditions as laid down in Directive 92/65. However, where harmonized rules for a particular species have not been laid down in the directive, national rules apply. The importing zoo must apply for a specific import

license, which will contain the conditions relevant to the species and place of origin.

Quarantine/Isolation Requirements

“Isolation” and “quarantine” are not precisely defined in EU legislation, and one word is usually described by reference to the other. For the purposes of adding animals from nonapproved sources within the EU and other countries where the “Balai” Directive applies, or from listed “Third Countries,” to an approved establishment, the requirements are therefore specified in the recommendations. The basic principle is that a risk analysis must be made, and the quarantine/isolation requirements must cope with the risk. Quarantine requirements for comparable livestock could provide some guidance. In this context, management procedures could be adjusted easily to each individual case, but the availability of suitable facilities is a prerogative for approval and must be seen without a specific case in mind, but considering that there are three main risk groups: primates, birds, and mammals (Figure 8-1).

Primates

Primate species may be imported from anywhere (there is no “Third Countries” list) and may be carriers



Fig 8-1 Border veterinary control station at Zurich Airport with isolation rooms allowing for the temporary keeping of animals that may transmit airborne diseases. (See Color Plate 8-1.)

of zoonoses. It is therefore recommended that the quarantine requirements in the World Organization for Animal Health (OIE) *Terrestrial Animal Health Code* (Chapter 2.11 and Appendix 3.5.1) be respected.⁴

Birds

The introduction of birds from areas where OIE List A diseases exist may not be excluded (occurrence of diseases in wild, in particular migratory birds), and the relevant diseases—Newcastle disease, avian influenza, and psittacosis—are easily transmitted by air or, in the case of West Nile virus, by mosquitoes. Birds must therefore be isolated in buildings, and the possibility of disease transmission by air or insects must be considered. Windows should be kept closed, and it is strongly recommended that the isolation rooms be ventilated and the exhausted air pass through a dust filter.

Mammals

Other than primates, under EU legislation the introduction of mammals is allowed only from areas free from highly contagious diseases. All diseases that are relevant in practice, such as rabies, tuberculosis, brucellosis, and bovine leukosis, are not transmittable by air over a longer distance. In most cases, direct contact is required. As a general rule, mammals other than primates should therefore be isolated indoors, but no special precautions have to be taken regarding the exhausted air to cope with the relevant diseases listed in Annex A of the “Balai” Directive. If, for specific reasons, mammals need to be isolated outdoors, the ground should be solid and easy to disinfect. If this is not possible, the isolation enclosure should be relatively small to allow for other treatment of the soil (e.g., removal of topsoil). No zoo will be able to have specific isolation facilities for all mammalian taxa, which may include a diverse range of species, including big cats, dolphins, elephants, and hippopotami. In such cases it should be possible to use the standard facility for isolation purposes.

Availability

The recommendations state that, to be granted approval, zoos must have *available* adequate quarantine/isolation facilities, and that this wording does not imply that the facilities are on the ground or owned by the zoo concerned. This allows for the option of several zoos jointly operating a facility, or having contracts among themselves, in which case the option should be specified in the annual plan.

Structural Specifications

The recommendations also provide guidance regarding the structural requirements of isolation quarters. These must be physically separated from other animal accommodations by a reasonable distance, taking into account the species involved and the ability of the relevant viruses to spread on the air. This distance may be greatly reduced if the exhausted air is filtered. For animals originating from within the EU or from listed “Third Countries,” the use of dust filters is sufficient, or high-efficiency particulate air (HEPA) filters may be required.

The limits of the isolation area must be clearly demarcated by walls or fences as appropriate. This does not preclude the possibility that specific areas or pens within the premises may be designated as isolation areas for a limited time and a particular purpose, provided that they meet the general requirements. There must be a double-door system to prevent escape at the entry/exit, with sufficient space between the doors to allow one to be closed before the other is opened. Entry/exit doors must be lockable and must display a notice stating, “QUARANTINE: No Admission to Unauthorized Persons.” Facilities must be available at the entry/exit point for attendants to change overalls, to change and disinfect boots, to wash hands, and if appropriate, to shower.

Suitable facilities must be available to load or unload animals between transport crates and isolation pens without the risk of escape. Suitable crush or penning facilities should be available within reasonable access of the isolation area so that animals may be safely restrained for clinical and diagnostic procedures (e.g., blood sampling). The route from isolation to restraint must not put other animals at risk of infection from the introduced animals. The design of the pens or cages within the isolation area must allow the animals to be visually inspected at any time, with adequate light and ease of access.

The physical structure and all equipment must be made of materials that can be effectively cleansed and disinfected, or destroyed after use. The design must be suitable to minimize access by rodents, wild birds, and insects, as appropriate for the species in question. Any drains must be fitted with rodent-proof covers. The feed store must be suitably protected from vermin. Adequate storage facilities must be available to contain the litter and animal waste produced during the isolation period, and the storage facility must be bird and vermin proof.

Facilities must be available to dispose of the waste during or after the isolation period in a way that will

ensure that there is no risk of disease spread. Refrigeration facilities must be available within the isolation area, or in a suitably disease-protected location nearby, to hold carcasses of animals until post-mortem examination. Procedures for conveying carcasses safely to the storage facility must be specified in writing by the approved veterinarian.

Management Procedures

Every animal in isolation must be visually inspected at least once a day by competent staff. Any signs of illness must be recorded and reported immediately to the responsible veterinarian, who should make a further examination of the affected animals without any unreasonable delay. The premises must have designated staff present on a sufficiently regular schedule to ensure surveillance of the animals on a daily basis, and more frequently if appropriate.

Staff entering the isolation premises must always change into protective clothing and footwear. On leaving, the overalls and footwear must be removed and left within the isolation area, and the footwear must be disinfected. Hands must be washed, or otherwise disinfected, on entering and leaving. None of the movable items used in the isolation unit should be taken outside the unit, or used with other stock outside the unit, for the entire duration of the isolation period. Litter and waste material must be collected regularly, stored in the containers provided, and disposed of either during or after the isolation period in a way that prevents spread of disease agents. Premises must have an effective program, specified in writing by the approved veterinarian, for cleansing and disinfection after each isolation session; approved disinfectants must be specified and used in the program; and an appropriate resting period must be specified after each cleansing and disinfection operation. Crates or cages used for transport, if reused, must be made of materials that allow effective cleaning and disinfection, which should be done within the isolation unit. If not reused, the crates and cages must be destroyed so that disease agents cannot be spread.

An “all-in, all-out” policy should be followed in the isolation unit. If it is necessary to add animals while others are already present in the unit, the isolation period for all of them must be extended until the latest completion date of any of the animals. If any animals become ill during isolation, and the approved veterinarian believes they need to be moved to a specialized hospital facility for diagnosis or treatment, the veterinarian must ensure that this is done under his or her personal supervision in such a way as to ensure no

possible risk of disease spread. In particular, the approved veterinarian must personally supervise the arrangements for maintaining isolation throughout the movement and for disinfecting any vehicles, rooms, and equipment with which the animal has had contact.

Any sign of disease or a death during isolation must be reported immediately to the approved veterinarian, who in turn must report immediately to the competent authority all suspicions of any infectious disease on Annex A and any deaths in isolation. Carcasses of animals that die during isolation, and if necessary, of those that are dead on arrival, must be submitted to a postmortem examination without unreasonable delay. The establishment must designate suitable staff to attend to the animals in isolation, taking appropriate precautions to ensure that there is no risk of transferring infection from the isolation unit to any other animals, and the arrangements must be agreed in writing by the approved veterinarian.

Visitors must not be allowed to enter the isolation unit. If personnel other than the designated attendants need to enter for essential maintenance, they must be required to wash thoroughly on entering and leaving and to wear protective clothing that is donned before entering and removed before leaving. There must be a visitors’ book to record the dates, names, and addresses of all visitors. The person in charge of the isolation unit must keep detailed records, which should be retained for at least 10 years.

Isolation should normally last for at least 30 days, unless a longer period is required to exclude specific risks such as rabies.

In addition to these general requirements, some specific recommendations are made for the isolation of birds, primates, and ungulates.

Certificates

The last chapter of the recommendations is brief, explaining which zoo animals require certificates other than those contained in Annex E of the “Balai” Directive.

CONSEQUENCES

Although the EAZWV *Transmissible Diseases Handbook*⁶ containing the text of the revised “Balai” Directive, the recommendations, and other relevant information was sent to all zoos, all national authorities, and in the case of Austria, Germany, and Switzerland, also state

veterinary authorities, not much changed for a time. Zoos were reluctant to be among the first to apply for approval, and within the veterinary services, information was often not transmitted to the official veterinarians directly responsible for supervising a zoo. As of autumn 2005, I was aware of only one approval in Austria, 17 in Germany, three in Switzerland, and 14 in the United Kingdom and brought this to the attention of the EU Commission, who subsequently urged the national veterinary services to go ahead with the approvals by making use of the EAZWV recommendations.

A considerable number of applications are currently pending. We now may anticipate that in the near future the "Balai" Directive will serve its purpose: to facilitate animal movements between European zoos while ensuring a good animal health status of the collections involved.

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Color Plate 8-1 Border veterinary control station at Zurich Airport with isolation rooms allowing for the temporary keeping of animals that may transmit airborne diseases. (For text mention, see Chapter 8, p. 71.)

CHAPTER 9

Encephalomyocarditis Virus Infection in Zoo Animals

KAY A. BACKUES

ETIOLOGY

Encephalomyocarditis (EMC) is a viral disease of mammals caused by infection with *encephalomyocarditis virus* (EMCV), which belongs to the genus *Cardiovirus* in the family Picornaviridae. Picornaviruses are nonenveloped, single-stranded ribonucleic acid (RNA) viruses that are resistant to ether inactivation.¹⁸ Other members of *Cardiovirus* include Columbia SK, Mengo virus, and MM virus.²⁸ The different species of *Cardiovirus* are serologically indistinguishable from one another.²⁹

EPIDEMIOLOGY

Members of *Cardiovirus* are found worldwide and have been reported to cause disease in a wide variety of mammals and, occasionally, humans.²⁹ Free-ranging and captive wildlife populations have experienced outbreaks of EMC.¹⁰⁻¹² Mortality has been reported in, but should not be considered limited to, the following families and orders: Suidae, Proboscidea, Pongidae, Cercopithecidae, Antelopidae, Camelidae, Tapiridae, Lemnidae, Cebidae, Rodentia, and Marsupialia.^{4,10,12,22,28} The infection is acquired through fecal-oral transmission. Rodents are thought to be vectors of the disease.^{27,29} In reported zoo outbreaks, a low level of virus isolation was found in rodents tested.^{22,28} However, the virus is efficiently transmitted horizontally and shed in the feces without morbidity or mortality in rats (*Rattus norvegicus*).²⁵ The presence of rodents and/or their feces appears to be the most important factor associated with reported outbreaks in both wild and captive populations.^{10,11,22,28}

Although EMCV is considered pandemic, outbreaks in U.S. zoos are primarily seen in states bordering the Gulf of Mexico, with no seasonality.¹ The association between disease and this subtropical region of the United States is not understood but may have contributing factors, such as vector species and

density, virus durability because of temperature, humidity, or other, unknown factors.

PATHOGENESIS

The replicative cycle of picornaviruses is rapid, completing in approximately 8 hours.²³ Pathogenesis of the genus *Enterovirus* is a model for the rest of Picornaviridae.^{18,23} The poliomyelitis enterovirus shows initial attachment by cell surface receptors and replication in the pharynx and gastrointestinal tract.²³ The poliovirus travels from regional lymph nodes to other reticuloendothelial organs through a minor viremia. Two outcomes are then possible: the host's immunity controls the infection, or a major viremia ensues, taking the virus to its target tissues, where it will precipitate clinical signs of disease.²³ The viremia of EMCV is thought to arise in a similar manner.²⁷ The target tissue of cardioviruses in nonrodent mammals is primarily the heart. Replication in cardiac muscle cells leads to severe acute myocardial cell death, failure of the electrical conduction system, and acute clinical signs of myocardial failure.

DIAGNOSIS

Antemortem Diagnosis

Animals may show subtle nonspecific clinical signs, such as lethargy and reluctance to move, but the typical clinical presentation is death without premonitory signs.^{4,11,22} Several animals in captivity have been found to have naturally acquired EMCV serum neutralization (SN) titers, indicating that exposure has caused subclinical infections. In one outbreak, three African elephants in a Florida facility had EMC virus SN titers greater than 1000:1. Reexposure to the virus may have been ongoing in these elephants, but the confirmed

outbreak of the disease in this institution was 20 years earlier.^{5,24}

Postmortem Diagnosis

Gross lesions of the disease are primarily limited to the cardiovascular system. Severe pulmonary congestion with blood-tinged foam in the trachea is a common finding.^{10,22} The lungs may weigh several times their normal weight because of the marked fluid accumulation. The myocardium is severely marked with pale streaks, and petechiae or ecchymoses on the epicardial surface maybe present (Figure 9-1). Other organs may or may not show gross signs of congestion.

Histologic lesions of EMCV infection in wildlife species are confined to the cardiovascular system.^{10,11,22,28} Lymphocytic, plasmacytic necrotizing myocarditis is seen in the myocardium. Lungs show marked edema and congestion.^{11,22,28} Encephalitis is more frequently seen in rodents and was reported in a bonobo.^{14,27,29} This lack of histologic findings of encephalitis may result from the infrequency with which central nervous system tissue is submitted for histopathology in larger animals. These findings, along with gross lesions and typical clinical presentation, are sufficient for a clinical diagnosis of EMCV infection.

Isolation of EMCV is not difficult and should be attempted to confirm the clinical diagnosis of EMCV infection. Tissues may be collected at necropsy and frozen for later viral isolation. The organs of preference for virus isolation include heart muscle, spleen, and brain in wildlife species and also liver and intestines in rodent species.²² A reference laboratory that

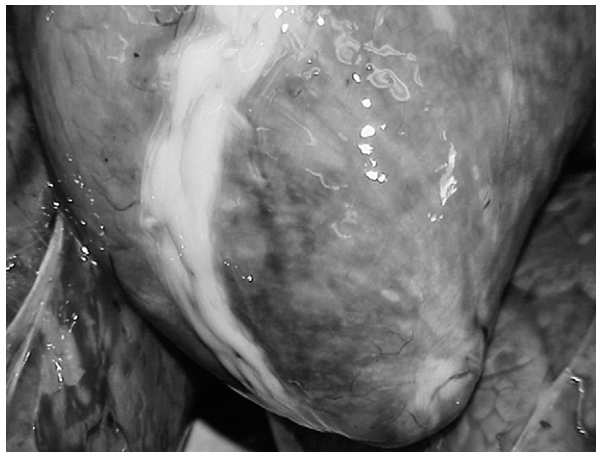


Fig 9-1 The heart of a guanaco that died acutely of EMCV infection. The epicardial surface of the heart shows multifocal pale streaking and petechiae. (See Color Plate 9-1.)

performs EMCV isolation should be contacted for sample submission.

PREVENTION

The cornerstone to prevention of EMCV infection in captive wildlife is rodent control and hygienic feeding practices. Veterinarians and wildlife professionals who have worked in captive or free-ranging situations during an EMCV outbreak have often noted an increased number of rodents, evidence of affected animals eating rodents, or fecal contamination from rodents.^{4,10,22,28} Additionally, zoologic and research facilities have reported the lessening or cessation of the outbreak when rodent control measures were undertaken.^{12,21} Rodent harborage in and around animal enclosures should be eliminated. The safe and judicious use of rodenticides is warranted. Food bowls should be cleaned and disinfected daily. The presence of rodent feces in food bowls and animal areas should be brought promptly to the attention of animal managers and veterinarians. Whenever possible, food bowls or troughs should be emptied at the end of the day to discourage feral rodents from sharing meals and defecating in feed bowls of captive wildlife. The timing of when zoo animals were offered food and when food bowls were removed was one of the changes instituted that helped stop EMCV deaths in primates and New World camelids at the Audubon Zoo in 1996.²

The general cleanup and disinfection of an affected enclosure and the surrounding area should be performed. Enclosure surfaces and food bowls should be cleaned with disinfectants that are capable of killing other picornaviruses, such as foot-and-mouth disease (FMD) (Table 9-1).¹⁵

The virus has the ability to survive in moist soil for 13 months.²⁵ Soil and substrate heavily contaminated with rodent feces should be removed from the enclosure.

Rodent control is an ongoing program in captive-wildlife facilities. The multidimensional and naturalistic enclosures needed to meet the needs of captive wildlife will always attract feral rodents. Once cleanup and control are instituted, these must become part of the daily routine of animal staff to remain a successful preventive measure.

Vaccination

An efficacious vaccine would be an important second step to preventing EMCV-related deaths in captive

Table 9-1

Disinfectants* Used for Cleanup of EMCV-Contaminated Surfaces

Product	Dilution	Mixing Instructions	Notes
5.25% sodium hypochlorite (NaOCl), household bleach	3%	Add 3 gallons (gal) bleach to 2 gal water; mix thoroughly.	
Acetic acid	4%-5%	Add 6.5 oz glacial acetic acid to 1 gal water; mix.	Vinegar is 4% solution of acetic acid.
Potassium peroxymonosulfate and sodium chloride	1%	Follow label directions.	
Sodium carbonate (soda ash)	4%	Add 5.33 oz NaCO ₃ to 1 gal hot water; mix.	Solution is mildly caustic.
Sodium hydroxide (NaOH) Lye (NaOH with soda ash)	2%	Add 1/3 cup NaOH pellets (2.7 oz) to 1 gal cold water; mix.	Solution is highly caustic; use protective rubber clothing, gloves, and safety glasses. <i>Always add the lye to the water.</i>
Formaldehyde	10%-30%	Not typically used on surfaces.	Highly tissue toxic.
Ethylene oxide (C ₂ H ₄)O	Gas	Used for sterilizing instruments.	Irritant to skin; toxic gas.

From *Disinfectants for foot and mouth disease—field use*, USDA/Aphis.gov.

*Listed for use against another picornavirus infection, foot-and-mouth disease.

wildlife. The relationship between protection from infection and the presence of neutralizing antibody titers to other picornaviruses (e.g., FMD, poliomyelitis virus, rhinovirus) is well documented.^{6,17} The presence of detectable levels of circulating antibodies to EMCV has coincided with survival of laboratory animal species and African elephants challenged with virulent EMCV.^{19,20} Several different vaccines, both live and inactivated, have been developed and used with variable results for inducing increased SN titers.^{4,8,9,13,16,20}

A vaccine trial of rhesus macaques (*Macaca mulatta*) using two different isolates of EMCV yielded unequal SN antibody titers.⁸ Ideally, to be considered successful, an EMCV vaccine would be safe, have a reasonable shelf life, and induce at least a fourfold increase in SN titer, and the resulting titer would persist for at least 12 months in multiple susceptible species. Virulence challenge to determine success of vaccination is seldom attempted because of the value of captive animals in facilities.^{4,16}

Currently, such a vaccine is not available for use by zoo and wildlife veterinarians in the United States. Lack of a commercially viable market for EMCV vaccine has hindered access to an “off the shelf” product. However, research with an experimental vaccine made with EMC viral isolates from two captive-wildlife facilities is ongoing.³ If shown to be safe and efficacious, this vaccine may become available on an experi-

mental basis to zoo and wildlife veterinarians in the United States.

Autogenous vaccines have been manufactured and used successfully in other countries.^{16,20} Continuing research and long-term tracking of SN titers is needed to determine vaccine makeup, EMCV isolate, and booster schedule for effective vaccination of susceptible species.

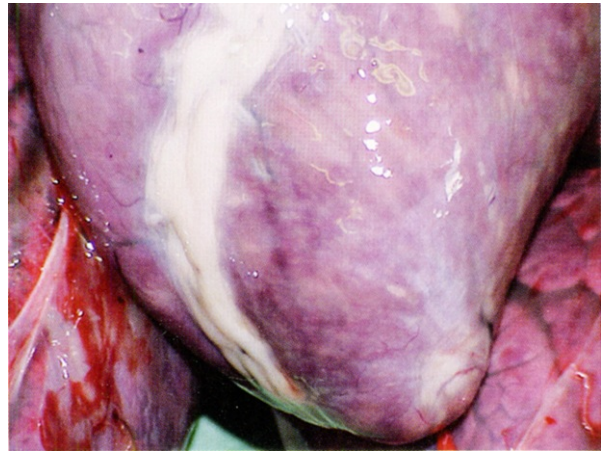
For highly valuable and susceptible species such as African elephants, determination of the animal’s baseline EMC virus SN titer may be an important management tool to determine level of exposure in a collection and susceptibility to infection of an individual before management transfers into regions with high EMCV prevalence.

ZOONOTIC POTENTIAL

Infection of humans by EMCV is common, with seroprevalence rates up to 50% of the adult population documented in some countries.²⁶ Most infections are asymptomatic and go unrecognized. However, caution is warranted with the handling of EMCV-infected tissues and working in contaminated areas because lethal EMCV infections are well documented in great apes.^{7,14,22} General universal precautions should be followed to protect staff from possible EMCV infection.

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Color Plate 9-1 The heart of a guanaco that died acutely of EMCV infection. The epicardial surface of the heart shows multifocal pale streaking and petechiae. (For text mention, see Chapter 9, p. 76.)

CHAPTER 10

Avian Influenza

JOOST D. PHILIPPA

DEFINITION AND TYPES

Avian influenza A virus (AIV) is a member of the Orthomyxoviridae family and may be classified according to the antigenicity of its surface proteins *hemagglutinin* (H) and *neuraminidase* (N), and on the basis of its pathogenicity in chickens after intravenous inoculation. Currently, 16 H (H1-H16) and 9 N (N1-N9) subtypes have been described in avian species.¹⁹ Individual subtypes may be composed of any combination of one of the H and one of the N proteins.

Highly pathogenic avian influenza (HPAI, formerly “fowl plague”), an acute, generalized disease in which mortality may be as high as 100%, is restricted to subtypes H5 and H7, although not all viruses of these subtypes necessarily cause HPAI. All other AIV strains are *low-pathogenic avian influenza* (LPAI) virus strains and cause a much milder, primarily respiratory disease with loss of egg production.⁷ In certain cases the LPAI virus phenotype (of subtype H5 or H7) may mutate into the HPAI virus phenotype by the introduction of basic amino acid residues (arginine or lysine) at the cleavage site of the precursor hemagglutinin (HA0),³ which facilitates systemic virus replication. H5 and H7 subtypes with an amino acid sequence at the HA0 cleavage site comparable to those that have been observed in virulent avian influenza viruses are considered HPAI viruses, even when mortality in chickens is low. However, the two forms of avian influenza (HPAI and LPAI) are distinctly different and should be regarded as such.

ETIOLOGY AND EPIDEMIOLOGY

Avian influenza virus has a worldwide distribution and is infective for all avian species (commercial, domestic, and wild), with variable morbidity per virus isolate and species. Aquatic avian species, mainly those of the taxonomic orders Anseriformes (ducks, geese, and swans) and Charadriiformes (shorebirds,

gulls, and terns), are considered the main natural reservoir of all avian influenza viruses, including the LPAI ancestral viruses of HPAI strains.³² Replication of LPAI viruses occurs mainly in the intestinal tract, with excretion of high virus loads, and efficient transmission via the fecal-oral route.⁵⁵ Experimentally infected mallards have been shown to excrete virus asymptomatically for up to 17 days.⁵⁹ It has been shown that AIV remains infectious in feces for up to 32 days¹⁷ and in lake water for 4 days at 22°C, to more than 30 days at 0°C.⁵⁵ Migrating waterfowl are thought to carry LPAI viruses over long distances and may initiate outbreaks of HPAI by the introduction of these LPAI viruses into poultry flocks, which subsequently change into HPAI viruses.⁵⁶ Recent HPAI H5N1 viruses have been predominantly associated with oropharyngeal shedding^{4,45}; the impact of this on environmental contamination, persistence, and transmission is still unknown.

Poultry species (e.g., chickens, turkeys, quail, ostriches) are generally highly susceptible to infection with HPAI virus. In 2002 an outbreak of HPAI H5N1 virus occurred in wild migratory avian species and resident waterfowl, and the high pathogenicity in ducks was confirmed in laboratory infections.⁴⁴ Since 2002, this particular HPAI virus subtype has made an unprecedented spread from Southeast Asia throughout Asia and into Europe and Africa, with morbidity and mortality not only in domestic poultry, but also in a large number of avian species, specifically, 96 species (spp.) from 14 orders: Anseriformes (31 spp.), Charadriiformes (5), Ciconiiformes (6), Columbiformes (3), Falconiformes (9), Galliformes (9), Gruiformes (4), Passeriformes (18), Pelecaniformes (2), Phoenicopteriformes (1), Strigiformes (4), Struthioniformes (1), Psittaciformes (1), and Podicipediformes (2 spp.).⁵² Outbreaks along the recognized flyways from Southeast Asia into Europe have suggested that this HPAI virus subtype may be distributed directly by migrating waterfowl, and HPAI virus infections have been detected in several migratory species.^{10,30,62} However, it remains clear that domestic

waterfowl,^{9,24,45} specific farming practices, agro-ecologic environments, and transportation of domestic avian species or their products with trade at local markets have played key roles in the amplification and spread of HPAI H5N1 virus in Asia.^{21,42}

Several mammalian species (ferrets, horses, pigs,⁹ seals, and humans^{13,18}) had been reported with infections with the H5 and H7 subtypes of AIV up to 1997. The recent HPAI H5N1 virus subtype has caused mortality in (experimentally) infected Owston's banded palm civets (*Chrotogale owstoni*),³⁹ domestic dogs,⁴³ domestic cats (*Felis domestica*),^{29,38} leopards (*Panthera pardus*) and tigers (*Panthera tigris*),^{28,50} stone martens (*Martes foina*), and ferrets (*Mustela putorius furo*)⁵² and has caused 256 human cases with 151 deaths to date (October 2006).⁶¹

Noteworthy are the fatal HPAI H5N1 virus infections with severe pneumonia of domestic cats, tigers, and leopards that fed on infected poultry,²⁸ because felids had previously been considered resistant to disease after AIV infection.²³ Horizontal spread of infection was suspected⁵⁰ and has been demonstrated experimentally in domestic cats,²⁹ with excretion from both the respiratory and the intestinal tract.³⁸

Fatal infections of domestic dogs with an H3N8 strain usually found in horses have been documented recently, and this virus is apparently spreading and has potential to become endemic in dogs.¹²

CLINICAL SIGNS

Low-pathogenic AI infections of wild birds usually produce no clinical signs. In poultry, LPAI is characterized by mild to severe respiratory signs, excessive lacrimation, decreased egg production, and signs of generalized malaise (ruffled feathers, depression, decreased water and feed intake). Disease caused by HPAI virus infection may vary depending on species, age, strain of virus, and environmental factors. Infections of poultry by HPAI viruses are characterized by a drop in egg production, inappetence, depression, respiratory signs, sinusitis, watery diarrhea, excessive lacrimation, edema of comb and wattles with cyanosis and hemorrhages, neurologic signs, and high mortality. Other avian species may have a higher resistance to infection, with no clinical signs shown, death soon after showing signs,⁵³ or slow recovery until no clinical signs are seen.⁴

AIV infection in mammalian species is predominantly an upper respiratory tract infection with some lung involvement. Signs of infection include sneezing, nasal discharge, malaise, and pyrexia. HPAI H5N1

virus-infected wild felids and laboratory cats have shown additional signs of respiratory distress, serosanguineous nasal discharge, protrusion of the third eyelid, conjunctivitis, neurologic signs, and death.^{28,29,38,50}

Postmortem Lesions

Gross lesions are variable, depending on host species, pathogenicity of the virus, and presence of secondary pathogens. Whereas lesions caused by LPAI in poultry are mostly found in the respiratory tract (especially the sinuses) and coelomic cavity (egg-yolk peritonitis), lesions caused by HPAI are more diverse. If death is peracute, no gross lesions may be observed. In poultry, subcutaneous edema of the head, upper neck, and feet may be accompanied by hemorrhages and cyanosis, particularly in the wattles and combs. Hemorrhages may also be found in visceral organs, especially the epicardium, pectoral muscles, and the mucosa of the proventriculus and ventriculus. Necrotic foci are most common in the pancreas, spleen, and heart. Lungs have focal ventral to diffuse interstitial pneumonia with edema and may be congested or hemorrhagic.⁴⁶

Viral-induced lesions in HPAI H5N1 virus-infected ducks and gulls were only found in those with clinical signs. Lesions found (petechial hemorrhages in pancreas, ventriculus, apex of heart, and cerebrum) were milder in birds that recovered.⁴

Histologic lesions consist of multiorgan necrosis and inflammation (mainly brain, pancreas, heart, lung, and primary and secondary lymphoid organs).^{4,11,46} The most common lesions in recovered birds are lymphoplasmacytic perivascular encephalitis and heterophilic pancreatitis.⁴

Surprisingly, experimental H5N1 virus infection in cats was demonstrated to be systemic and not limited to the respiratory system. Gross lesions consisted of congestion of the lungs and enlargement of, with multifocal petechial hemorrhages in, the tonsils, mandibular lymph nodes, retropharyngeal lymph nodes, and liver.³⁸ Infected tigers grossly had severe congestion of the lungs with hemorrhages, serosanguineous exudates throughout tracheal and bronchiolar lumina, and pleural effusion. Histologic lesions consisted of severe diffuse lung hemorrhages and edema, nonsuppurative meningoencephalitis, and moderate multifocal necrotizing hepatitis^{28,29,38,50} (Figure 10-1). Extrarespiratory spread in laboratory cats included tissues of the nervous, cardiovascular, urinary, digestive, lymphoid, and endocrine systems and was associated with severe pathologic changes, consisting of necrosis and inflammation.³⁸

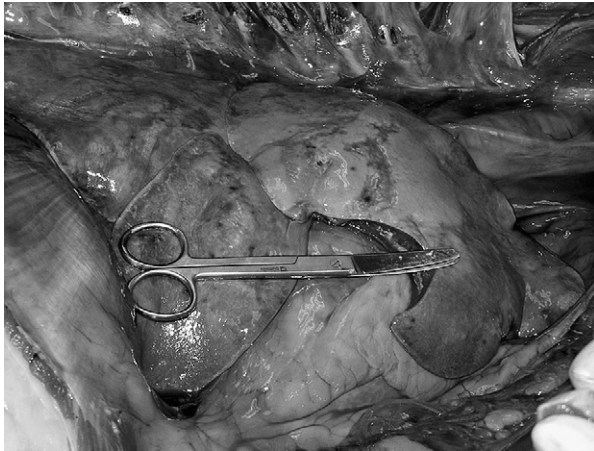


Fig 10-1 Tiger lung at necropsy showing hemorrhages and severe pulmonary consolidation. (See Color Plate 10-1.) (Courtesy Rattapan Pattanarangsarn, Mahidol University, Salaya, Nakorn Pathom, Thailand.)

DIAGNOSIS

The definitive diagnosis may only be made after viral ribonucleic acid (RNA) antigen detection by polymerase chain reaction (PCR), with subsequent nucleotide sequencing, or by isolation and characterization of the virus. The range of nonpathognomonic clinical and pathologic manifestations produced by influenza viruses, and the detection of AIV-specific antibodies, may only lead to a presumptive diagnosis.

Material for Laboratory Analysis

Cloacal and oropharyngeal swabs may be taken to show presence of virus or viral RNA in live birds. Small birds may be harmed by swabbing, and for these species, collection of fresh feces is an adequate alternative. Cotton swabs should be placed in vials with virus transport medium⁶⁰ (e.g., Hank's balanced salt solution with 10% vol/vol glycerol, 200 U/mL penicillin, 200 µg/mL streptomycin, 100 U/mL polymyxin B sulfate, and 250 µg/mL gentamicin, at a final pH of 7.0-7.4) and kept on wet ice. Samples may be stored at 4°C for up to 4 days; prolonged storage should be at -80°C.

Postmortem samples (intestinal contents; cloacal and tracheal swabs; trachea, lung, intestine, proventriculus, pancreas, spleen, kidney, brain, liver, heart) of animals should be collected for virus isolation and stored at -80°C or preserved in a lysis buffer for RNA isolation and reverse-transcriptase PCR (RT-PCR). Organ samples should also be fixed in 10% neutral-buffered formalin and embedded in paraffin for histology and immunohistochemistry.

Suspensions in antibiotic solutions of organ material, swabs, or fecal material may be inoculated in embryonated chicken eggs, which are incubated and tested for hemagglutinating activity. The conventional isolation in chicken eggs and characterization is still the method of choice for the World Organization for Animal Health (OIE),³⁴ but molecular techniques for the detection of viral RNA by RT-PCR techniques have been developed, which are much faster, generating reliable subtype-specific results within a few hours, and may provide complementary (copy) deoxyribonucleic acid (cDNA) for nucleotide sequencing.³⁴

A human rapid enzyme immunoassay (EIA) kit (Directigen Flu-A, Becton-Dickinson Microbiological Systems, Alphen aan den Rijn, Netherlands), which uses a monoclonal antibody to detect the nucleoprotein of influenza A strains, has been reported to detect viral antigen in avian specimens as well.⁴⁰ This test takes 20 to 30 minutes and therefore might be useful for screening. However, the test lacks sensitivity, has not been validated for different avian species, and subtype identification is not achieved.

Serum may be collected for the detection of antibodies to nucleocapsid or matrix antigens by means of agar gel immunodiffusion tests. These antigens are antigenically similar in all influenza A viruses, but not all birds develop detectable titers.³⁴ Hemagglutination inhibition (HI) tests, which are subtype specific, may be used for routine diagnostic testing, for epidemiologic studies, and to determine vaccine efficacy.

Differential Diagnosis

Differential diagnoses of HPAI in wild avian species include acute toxicoses (e.g., botulism, algal bloom), Newcastle disease and other paramyxoviruses, pasteurellosis or other septicemic bacterial infections, duck viral enteritis (duck plague), West Nile virus (WNV) and other flaviviruses, equine encephalitis, and infectious laryngotracheitis.

MANAGEMENT, THERAPY, AND PREVENTION

Highly pathogenic avian influenza (HPAI) viruses, but also H5 and H7 LPAI, are categorized as notifiable diseases by the OIE, based on their potential for rapid spread, serious economic or public health consequences, and impact on the international trade of animals and animal products. Most countries have banned vaccination of domestic poultry because of interference with

eradication policies and international trade agreements, but they will allow regulated vaccination in emergencies. Eradication measures during an outbreak in domestic poultry include (long-term) confinement, stamping out of all poultry on the infected farm, preemptive culling of animals on neighboring farms, and emergency vaccinations (European Union [EU] Directive 92/40/EEC).

Surveillance of wild birds may provide early warning signs for the introduction of HPAI virus.³³ Several countries have initiated surveillance campaigns of free-ranging wild birds. Wild bird populations that experience high mortality rates should be submitted to national or regional reference laboratories for testing (for a European listing, see the *EAZWV Handbook of Infectious Diseases*²⁰). Birds showing clinical signs may be captured, isolated, and selectively culled when testing is positive for HPAI virus. There is no scientific basis for large-scale culling of free-ranging wild bird populations to control outbreaks or their spread, and it would be highly undesirable from a conservationist perspective.³⁶ Instead, measures should be taken to prevent poultry from coming into contact with wild birds.

In zoos a balance should be found between what control measures are effective, practical and realistic, and socially tolerable. The standard measures used in poultry would be detrimental to the welfare and breeding programs of valuable and endangered avian species in zoos. Increased biosecurity is the first line of defense during outbreaks of HPAI and may be complemented by vaccination. Accreditation of zoos (e.g., by AZA, EAZA, or other national/international organizations) has resulted in standardized high levels of biosecurity, decreasing the risk of introduction and increasing the likelihood of containment of infectious diseases. With an outbreak of HPAI, however, levels of biosecurity should always be raised immediately, with hygienic measures implemented accordingly, to prevent entrance or spread of the virus. Attention should focus on both *exclusion* (identification and elimination of possible routes of entrance, e.g., by live birds, cages, equipment, clothing) and *containment* (reduction of the risk of infection for neighboring cages) of the virus. Detailed guidelines have been written by the Association of Zoos and Aquariums (AZA)¹ and the Wildlife Conservation Society (WCS).⁵⁸

In short, during an HPAI outbreak, no movement of susceptible animals should occur into or out of the zoo. Disinfectant footwear baths should be placed at the entrances/exits of the facility and enclosures. Infected animals should be culled or quarantined, monitored for viral shedding, and treated with appropriate antiviral drugs in certain cases. Animals that die

should be sent to official veterinary authorities for postmortem examinations and testing. In-contact birds should be isolated, observed, and tested regularly. Separate cleaning utensils, tools, and feeding bowls should be used for each enclosure. Wild birds must be prevented from flying into enclosures, which should be shielded to prevent wild bird droppings from falling in, where possible. Derogations to biosecurity measures (e.g., alleviation of confinement measures) may be made in zoos, especially when birds are vaccinated (European Commission [EC] Decision 2005/94/EC), provided that such derogations do not endanger disease control.

The combination of stringent hygienic measures, selective culling, isolation and antiviral treatment of infected animals, disinfection of the area, and vaccination of birds has proved effective in curtailing AIV outbreaks in zoos in Southeast Asia.

Disinfection

Hygienic practices are the first line of defense during epidemics of HPAI. As previously stated, AIV remains viable for long periods in feces (32 days) and water (4 days at 22°C, more than 30 days at 0°C). Simple cleaning with water without a detergent may result in spreading of virus through footwear, clothing, cages, equipment, and other fomites. It is essential to thoroughly disinfect items that have been in contact with bird feces or other secretions.

AIV is easier to inactivate than many viruses. Orthomyxoviridae contain an outer lipid layer that enables the virus to enter animal cells. This layer is very sensitive to detergents, and therefore soap and detergents are effective for disinfecting most items. Disinfectants should keep surfaces thoroughly wet for a contact time of at least 10 minutes.

Other means of virus inactivation include the following^{2,17}:

- **Oxidizing agents:** Chlorine is an effective broad-spectrum disinfectant but loses effectiveness in the presence of organic materials. Potassium peroxy-monosulfate combined with sulfamic acid, malic acid, sodium hexametaphosphate, sodium, and dodecyl benzene sulfate* has proved to be very effective under demanding farm conditions with short contact times and heavy organic challenge.

*The Dutch National Inspection Service for Livestock and Meat (RVV) recommended a 1:320 dilution of Virkon (DuPont) during the 2003 outbreak of HPAI H7N7 virus.

- **Alkalis:** 2% Sodium hydroxide (caustic soda) and sodium carbonate (washing soda) are highly effective, also under heavy organic burden, and are therefore ideal for disinfection of animal housing, drains, waste pits, and so forth, but should not be used on aluminium or similar alloys.
- **Acids:** Inactivation is rapid at pH 5.0. Citric acid (0.2%) is safe for use on clothes and body and requires 30 minutes of contact time.
- **Formaldehyde gas:** Because of its high toxicity, formaldehyde gas should be used to disinfect rooms or machinery only if there is no alternative. It requires 15 to 24 hours of contact.
- **Temperature:** The virus is inactivated at 56°C applied for 3 hours, or at 60°C for 30 minutes. Conventional cooking practices, in which core temperatures of 70°C are reached (and the meat is not pink in any part), are therefore sufficient, and consumption of properly cooked poultry is safe.²⁵

Vaccination

Vaccination should be considered in the face of an AIV outbreak, especially in flocks where management systems preclude birds being permanently housed indoors (EC Decision 2006/147/EC) and where depopulation is not feasible or desired (e.g., in zoos).

Vaccination is a useful means of reducing the horizontal spread of AIV in poultry,^{7,54} although in most countries it may be used only in emergencies and requires governmental approval. Nonetheless, prophylactic vaccination programs have previously assisted in the control of outbreaks of HPAI H5 and H7 viruses in poultry in Pakistan, Italy, Mexico, El Salvador, and Guatemala. Also, Southeast Asian countries have used vaccination as part of their control strategies against the recent HPAI H5N1 virus outbreaks. An inactivated vaccine (Nobilis Influenza H5, Intervet International, Boxmeer, The Netherlands), using an H5N2 strain (A/Chicken/Mexico/232-CPA/94) proved efficacious in chickens in Hong Kong under field conditions and after high-dose laboratory challenge by HPAI H5N1 viruses, with the protective effect becoming apparent 18 days postvaccination.¹⁶ Furthermore, other inactivated vaccines, H5N1 reverse genetic vaccines, and fowlpox recombinant vaccines with H5 inserts have been shown to be protective in chickens,^{48,49} ducks,^{31,57} and geese⁵¹ against diverse HPAI H5N1 virus strains.

Vaccination protects against disease and mortality but does not always prevent infection. However, the dose required for infection is much higher, and vacci-

nated birds shed much less field virus after infection than unvaccinated birds.^{5,27}

Antibodies produced in response to infection or vaccination are directed against the H and N surface proteins, and this response may vary between avian species, being higher in chickens than in other domestic species.²² Vaccine-induced protection depends on the species, dose, and vaccine strain. Published protective serum antibody titers measured by HI test in chickens postvaccination are 1:10,⁴⁶ or 1:16.^{16,51} However, ducks with very low or undetectable antibody titers postvaccination have been shown to be protected from HPAI virus challenge.^{31,57} The degree of homology of the H protein will affect the level of cross-protection and therefore the efficacy of the vaccine.⁴⁷ A *differentiation of infected from vaccinated animal* (DIVA) strategy, with a heterologous vaccine (using the same H subtype as the field virus, but a different N subtype), is recommended⁶ to differentiate between vaccinated and field-virus-infected animals. However, in housing systems where birds are not housed permanently indoors (e.g., in zoos), contact with free-ranging birds may result in LPAI virus infections that go unnoticed, but that may interfere with the DIVA principle.

A correlation between antigen dose and protection has been described in chickens,¹⁵ although very low doses were sufficient for protection in ducks.⁵⁷ Chickens have been protected from HPAI virus challenge for up to 10 months after one dose of vaccine; ducks were protected for more than 52 weeks after two doses; and geese that received three doses were protected for 34 weeks.⁵¹

Vaccination of avian species in zoos was approved during an outbreak of HPAI H7N7 virus in The Netherlands in 2003 (EC Directive 2003/291/EC) under strict conditions. An inactivated vaccine (Nobilis Influenza H7N1, Intervet) was used and evaluated in 211 birds of 64 species, from 13 taxonomic orders (10% of the total number of birds vaccinated). After booster vaccination, 81% of all vaccinated birds had seroconverted to a titer of 1:40 or higher, overall geometric mean titer (GMT) was 190 (95% CI, 144-251), and there was 79.2% agreement between antibody titers against the field virus and the vaccine strain.³⁷ Vaccination was therefore considered effective and useful. A titer of 1:40 (instead of 1:16) was used as a measure of efficacy because of the possible interspecies variation and the inability to perform a challenge infection to prove protection. A negative correlation between antibody response and increasing mean body weight was seen, despite administration of a double dose in bird species with average weights greater than 1.5 kg (Figure 10-2).

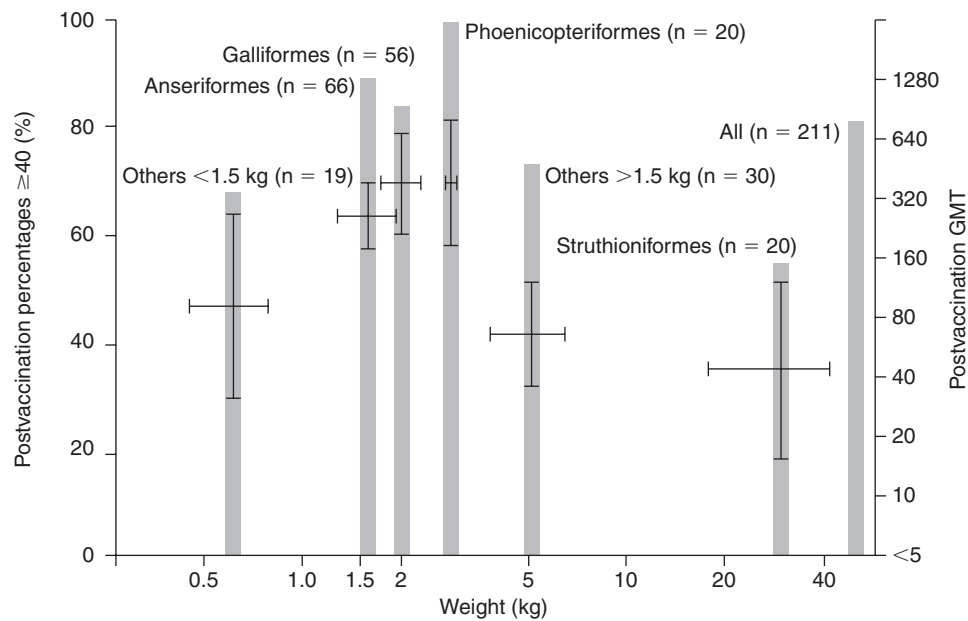


Fig 10-2 Humoral response of different avian orders to vaccination against avian influenza (H7) using an inactivated vaccine administered twice with 6-week interval. Titers shown were measured 30 to 60 days after the second vaccination. The blue bars represent the percentage of birds with titer of 40 or greater. The points represent the geometric mean titer (*GMT*) and mean body weight per order, with the 95% confidence intervals (*CI*) represented by vertical lines (*GMT*) and horizontal lines (mean body weight).

Vaccination of avian species against HPAI H5N1 virus was allowed in European zoos in 2005 (EC Directive 2005/744/EC) under similar strict conditions as in 2003. Vaccination of avian species in zoos using a commercial H5N2 vaccine, with vaccine doses adapted to mean body weight per species, was safe and proved immunogenic after booster vaccination throughout the range of species tested; some variations were seen among and within taxonomic orders. I further demonstrated the breadth of the immune response by measuring high antibody titers against prototype strains of four antigenic clades of currently circulating H5N1 viruses. Vaccination of avian species in Singapore Zoological Gardens using Nobilis Influenza H5 produced similar results. Titers measured 6 months postvaccination had dropped considerably but were still detectable in a large number of vaccinated birds.³⁵

A cause for concern is the (greatly reduced) replication and shedding of field virus that may still occur in the respiratory or gastrointestinal tract of asymptotically infected, vaccinated birds.⁷ These birds are likely to have a delayed diagnosis, resulting in spreading of virus and its potential for becoming endemic. To minimize the likelihood of virus circulating in the vaccinated population, vaccination should always be used in conjunction with strict biosecurity measures and monitoring for vaccine efficacy and shedding of field strain virus. Vaccinated birds that become

infected should be selectively culled or, in selected cases, quarantined and treated.

Currently, no commercial vaccine is available to protect mammals from HPAI H5N1 virus infection. A recombinant fowlpox-vectored vaccine expressing the H5 gene has been shown to produce high antibody titers against heterologous H5N1 virus antigen in cats after booster vaccination.^{38,26} It may prove useful in prophylactic vaccination programs of mammals in the future.

Vaccination of avian species in zoos should be regarded as a beneficial component of the preventive measures (including increased biosecurity measures and virologic monitoring) that may be undertaken to prevent an outbreak of, and decrease environmental contamination by, HPAI H5N1 virus while alleviating confinement measures.

Antiviral Drugs

Although culling is recommended for infected animals, isolation and treatment may be considered for certain valuable and endangered species. Large-scale administration of antiviral drugs in animals should be avoided to minimize the development of resistant virus strains.

Currently, two classes of antiviral drugs are available for use against influenza viruses: inhibitors of the ion channel activity of the M2 membrane protein of influenza A viruses (e.g., amantadine, rimantadine), and

neuraminidase inhibitors (e.g., oseltamivir, zanamivir), which are effective against influenza A and B viruses. Resistance to amantadine and rimantadine in influenza A viruses has been shown to occur rapidly during treatment.⁴¹ Both oseltamivir and zanamivir have demonstrated efficacy with minimal side effects in clinical trials. Because of a lower bioavailability, zanamivir must be inhaled, whereas oseltamivir may be given orally. However, the dosage, pharmacokinetics, and host metabolism have not been studied in vivo for most species, which may account for the failure of treatment with oseltamivir during an outbreak of H5N1 virus infection in tigers.⁵⁰ The emergence of oseltamivir resistance in H5N1 virus isolates from treated human patients¹⁴ is a cause for concern and may lead to an altered strategy for the treatment of influenza A infection.

ZOONOTIC POTENTIAL

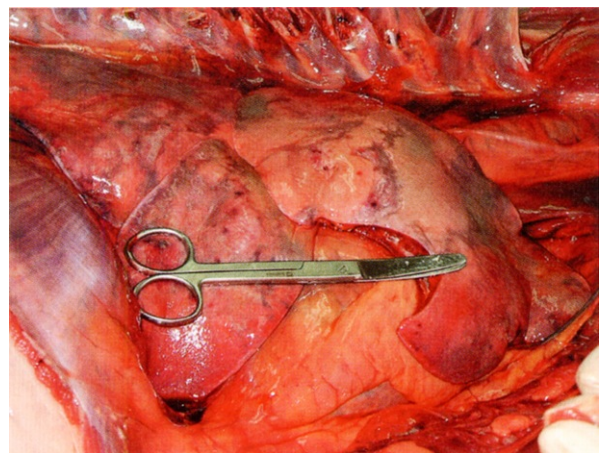
Avian influenza is a zoonotic disease, and H5 and H7 subtypes have caused clinical and fatal infections in humans.^{13,18} People who are or will be directly exposed to (potentially) infected animals or contaminated materials should be vaccinated with the seasonal WHO-recommended human influenza vaccine. Although this vaccination will not provide protection against infection by HPAI virus (H5N1), it will diminish the potential of simultaneous infection by a human and avian strain with possible genetic reassortment of the virus. Personal protective equipment, including disposable overalls, boots or disposable footwear, gloves, face mask, and goggles, should be worn. Hands should be washed frequently and disinfected after contact with animals. Exposed staff should be included in the surveillance (serology, virologic swabs, clinical signs). Antiviral drugs (e.g., oseltamivir) are recommended for people who have been in direct contact with HPAI-infected animals or contaminated materials.

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Color Plate 10-1 Tiger lung at necropsy showing hemorrhages and severe pulmonary consolidation. (For text mention, see Chapter 10, p. 81.) (Courtesy Rattapan Pattanarangsarn, Mahidol University, Salaya, Nakorn Pathom, Thailand.)

CHAPTER 11

Disease Management in Ex-Situ Invertebrate Conservation Programs

ROMAIN PIZZI

INVERTEBRATE CONSERVATION AND ZOOS

Animal conservation programs have traditionally been dominated by “charismatic” vertebrate species.¹⁸ The International Union for the Conservation of Nature (IUCN) has evaluated the conservation status of 100% of the 9932 avian species it currently recognizes and 99% of the 4842 mammalian species, but less than 0.3% of the over 1 million currently described invertebrate species have been evaluated. Although most authorities believe that the real number of invertebrate species is likely 6 to 10 million,^{15,24} others estimate the number may be as high as 80 million.^{56,57} Whatever the actual figure, we may not even be certain to within an order of magnitude of the true number of these species.^{22,23}

Conservation is usually defined as the “preservation of biodiversity,”²⁴ and the World Conservation Strategy¹⁷ proposed this as one of the three basic objectives of conservation. Ignoring invertebrate species in zoo programs could reduce the meaning of our work in fulfilling the definition of conservation. It is hoped that zoos will continue to expand their captive conservation programs to include a broader cross section of the animal kingdom, including invertebrates.³⁵

Although the educational use of invertebrates to illustrate the importance of preserving biodiversity probably remains their main contribution at present, zoos are also involved with some captive breeding of endangered invertebrate taxa for potential future reintroduction programs.^{13,36-38} Invertebrate captive breeding may be more economical than breeding of vertebrates because they have a shorter generation interval and higher yield. Therefore, invertebrates may be a convenient means of boosting a zoologic institution’s captive breeding contribution for minimal cost. Unfortunately, maintaining viable, self-replicating

captive colonies is often not sufficient to ensure the success of reintroduction programs.^{13,18}

Also, it remains to be seen how this definition of conservation, as the preservation of biodiversity, will affect wildlife veterinarians when dealing with parasites that may be even more endangered than their more charismatic hosts. There has already been debate on lethal side effects of relatively nonspecific treatments, such as the macrocyclic lactones, on minimally pathogenic but often extremely host-specific parasites, such as avian Mallophaga lice,³⁵ not to mention nontarget species such as endangered dung beetles. In the future the conservation biology community may criticize veterinarians’ sometimes indiscriminate use of these treatments.

In regard to zoo-based invertebrate conservation programs, unfortunately, disease risk assessment, screening, and management lag behind vertebrate programs. Therefore, zoo veterinarians need to develop increased interest and involvement in maintaining the health of this significant portion of the animal kingdom.

PLANNING ACCORDING TO EXTINCTION THREATS

Recognition of the specific extinction threat causing a population to decline in its native habitat is essential to the success of any invertebrate captive breeding and reintroduction program. Conservation plans, captive management, and pathogen risk limitation need to be planned with this in mind. The most important threats are habitat loss or change, overkill, exotic species invasions, chains of extinction (decline in one species leading to declines or extinction in a host of other interdependent species), and nontarget pesticide effects.²⁶ Most conservation entomologists believe that habitat loss or change is the main global threat to inverte-

brate biodiversity. Therefore, to be successful, captive breeding programs for reintroduction must be coupled with habitat conservation or modification programs.

As in charismatic megavertebrates such as elephants or rhinoceroses, unsustainable harvesting, or overkill, has also played a key role in the decline of a number of invertebrate species. Lepidoptera are particularly affected. Unfortunately, the more endangered the species, the more desirable it is to collectors. The British subspecies of the large copper butterfly (*Lycaena dispar dispar*) was collected to extinction by 1848. The numerous reintroduction attempts at sites in Britain and Ireland all have ultimately failed.^{9,26}

The black-veined white butterfly (*Aporia crataegi*) became extinct in the United Kingdom (U.K.) in the 1920s, but the reason for its decline could not be established. Although many reintroductions have been attempted, all these have again ultimately failed. This highlights the fact that captive breeding and reintroduction may be ineffective unless the underlying problem is identified and then corrected. Of the four species of butterfly to become extinct in the U.K. in the last 200 years, only the large blue butterfly (*Maculinea arion*), extinct in 1979, has been successfully reintroduced since 1983 using stock from Sweden. This butterfly has specific habitat requirements. Its larvae initially feed on the flower heads of wild thyme (*Thymus polytrichus*), but after the fourth instar, they feed on ant grubs within the nests of *Myrmica* red ants.⁵⁹ Accurate identification of the factors causing its extinction, as well as management of its habitat requirements as part of the reintroduction, has been essential to its success.

We are likely to see the increasing influence of genetically modified crops in the future, as well as the more well-documented effects of some pesticides on nontarget species. Genetically modified corn pollen has been shown to affect monarch butterfly larvae (*Danaus plexippus*) feeding on neighboring milkweed.²¹ Crops genetically modified to produce *Bacillus thuringiensis* toxins are likely to increasingly affect some nontarget invertebrate species, mainly those with a particularly alkaline midgut pH.⁵⁸

The introduction of exotic species has devastated some localized species, such as the Polynesian *Partula* species tree snails, many of which were decimated by the poorly conceived introduction of *Euglandina rosea* predatory snails, released as a postulated biologic control measure for introduced giant African land snails (*Achatina fulica*), which had become crop pests. Inadvertent introduction of a novel invertebrate pathogen into an already-threatened, naive wild population could be just as disastrous.⁶

PATHOGEN RISKS OF INVERTEBRATE REINTRODUCTIONS

As with vertebrate captive breeding and reintroduction programs, careful disease-screening measures are essential before invertebrate reintroductions.⁶² It would be irresponsible for zoologic collections to undertake breeding and release programs without pathogen risk assessments and screening to prevent the introduction of diseases into the wild population.¹ It is generally accepted that deliberate or accidental introduction of exotic organisms or diseases into ecosystems by humans has been one of the major causes of extinctions and loss of biodiversity in recent years.²⁷ Unfortunately, pathogen evaluations are often limited to investigation once an epizootic occurs. The IUCN guidelines for reintroductions¹⁴ and translocations¹⁶ are as relevant to invertebrates as to vertebrates.

One useful captive husbandry principle is to avoid having enclosures of species with high conservation priority adjacent to exhibit species of low importance. An example would be an endangered weta species (*Deinacrida*) enclosure next to a desert locust (*Schistocerca gregaria*) exhibit, a species that is often infected, even if subclinically, with eugregarines.³⁶ Although these parasites are usually regarded as fairly host specific, both insect species are members of the Orthoptera, and it would be sensible to keep these separated. Because some Eugregarines do not cause increased mortality and may only cause subtle signs such as reduced fecundity and reduced growth rates, monitoring of dead individuals may not be sufficient to detect infection in the population. Pooled feces in ethanol could be examined for spores. Infection may not clearly affect captive population with optimum environmental conditions and surplus food but could significantly impact the survival probability of the same species in its natural habitat. Implementation of similar principles is also recommended with different taxa, such as gastropod snails.

Marine invertebrates are often not quarantined or screened before introduction by aquarists, but introduction of pathogens by this route is well recorded.⁵³ Once introduced, disease control may be extremely difficult, with the complications of filtration systems and the sensitivity of exhibit invertebrates to many therapeutic agents.

Pathogen introduction risks are not only important to endangered invertebrate populations, but also to economically important insects. These include honey bees (*Apis mellifera*) and silkworms (*Bombyx mori*), as well as crop pollinator species. Some crops depend on a single insect species for their pollination, such as oil

palms (*Elaei guineensis*) pollinated by the weevil *Elaeidobius kamerunicus*.

Little literature exists on the diseases of economically unimportant invertebrates, which unfortunately includes conservation priority species.*

Additionally, novel infections are emerging in captive populations kept for conservation reasons.⁸ An invertebrate example is the recently described oral Panagrolaimidae nematode infection of tarantula spiders (Theraphosidae).[†] *Brachypelma* spp. such as the Mexican redknee *B. smithi* (listed in CITES Appendix 1) are popular zoo exhibit species. This fatal infection has been noted in captive bred terrestrial and arboreal species from the Americas, Asia, and Africa, including *Brachypelma* and *Poecilotheria* spp. The mode of transmission is unknown, but collections have reported spread of infection between separate enclosures in the same rooms, where spiders are obviously housed individually. Attempts at clinical treatment with benzimidazoles and antibiotics for concurrent bacterial infections have not prolonged survival.

The zoonotic potential of some related nematode species is also cause for concern. Mammalian infections have occurred as infections of deep wounds and are difficult to treat. Large species such as the Goliath bird-eating spider (*Theraphosa blondi*) may have fangs up to 3 cm in length, making infection of human bites a possibility. Because of the zoonotic risk and unsuccessful treatment attempts, euthanasia of affected spiders is strongly advised. With the emergence of this infection, it has been suggested that until the mode of transmission has been elucidated, veterinarians dealing with Theraphosidae spiders in zoologic collections institute a minimum quarantine period of newly acquired spiders, in a separate room, of at least 30 days. This should be prolonged in anorexic spiders approaching ecdysis (shedding), until resumption of normal feeding behavior after ecdysis has been observed.[†] This problem may adversely affect any future efforts at reintroduction and warrants further investigation.

The field of entomopathology is extremely well developed,^{2,19,58} but it has remained almost entirely focused on control of agricultural pest species and on human and domestic animal disease vectors. Modern techniques are often not applicable or affordable for disease screening in zoologic collections.⁵¹ The histologic basis of entomologic pathology research^{52,54,55} has now given way to molecular techniques, with the result that few entomologists are able to help with histopathologic queries.³⁷ Zoo veterinarians may be

familiar with disease screening in a large variety of vertebrate species but are usually limited in experience with invertebrate diseases and pathology.* Many of the most useful texts to a veterinary invertebrate pathologist are thus those from before this change in direction of entomologic pathology research.^{19,52,54,55}

EVALUATING THE SIGNIFICANCE OF INFECTIOUS AGENTS

The well-recorded extinction in captivity of the last *Partula turgida* Polynesian snails, has been claimed to be the first proven case of extinction by infection, with a *Steinhausia* species microsporidian infection implicated.⁷ Unfortunately, because no initial disease screening or archiving of wild individuals' tissues for later examination was performed, before captivity the true significance of this organism is by no means clear. It may have been present in the original wild population, even if at a low level.^{36,37}

A similar situation is infection with the entomopathogenic fungus *Metarhizium anisopliae* var. *anisopliae* of captive stock of the endangered Frégate Island giant tenebrionid beetle (*Polposipus herculeanus*). This beetle was listed as critically endangered after the introduction of rats to its home island in the Seychelles.¹⁰ The rats have since been eradicated, but the beetle still has a restricted range, making the population vulnerable to a stochastic threat. Although adult beetle mortality rates appear to be low,¹⁰ approximately 30% of all dead adults examined postmortem demonstrated infection. This isolate appears to have a relatively low pathogenicity in adult beetles, but it is unknown whether this agent occurs naturally on Frégate Island or in beetles in their natural habitat. It is thus impossible to determine sensibly the level of intervention and disease control needed in captivity. Although the risk of introducing a novel pathogen into a naive wild population is to be avoided at all costs, rigorous isolation, culling, and time-intensive husbandry techniques would be counterproductive should this agent occur naturally in the wild population. Further research is therefore essential before the reintroduction of any captive bred beetles could be considered.

The diagnosis of pathogens is also complicated by the occurrence of normal entomosymbiont organisms. These may be intracellular or extracellular and may occur in specific regions, such as the digestive tract, or may be free in the hemolymph. These entomosymbionts may be found in highly developed structures or mycetomes,⁵⁵ which have specific physiologic,

* References 4, 5, 25, 28, 29, 44 46, 63.

† References 34, 40, 41, 44, 45, 47.

* References 3, 4, 12, 29, 60, 61.

organlike functions in some species. Mycetomes may contain a combination of several different organisms. *Blattaria* spp. such as the commonly kept Madagascan hissing cockroach (*Gromphorhina portentosa*) have mycetomes associated with their adipose bodies. These are believed to be bacterial and rickettsial in nature. Elimination of the symbionts leads to abnormal protein metabolism, shortened survival rate, and reproductive failure.⁵⁸ *Partula* spp. Polynesian tree snails appear to have large numbers of normal flagellates, which do not appear to be associated with disease.⁴⁸ If pathologists are not familiar with the species, these may be erroneously diagnosed as pathogens.

PRACTICAL MANAGEMENT OF PATHOGEN RISK IN EX-SITU BREEDING PROGRAMS

Disease screening does not need to be time consuming, expensive, or complicated, but well-devised and standardized protocols are necessary. An initial broad-base screening of a sample of the population and sample archiving when first taken into captivity or a collection are always the ideal. Establishing a baseline of normal histology, microbial fauna, endosymbionts, and mycetomes in a species may help ensure rapid, cost-effective disease monitoring. This has not been done in practice, however, and has made diagnosis of disease outbreaks difficult, as in the previously mentioned cases of *Partula* snails and Frégate Island beetles.

If initial screening of a population is not possible because of the lack of expertise, time, or funds, it is still essential that a proportion of the population be preserved as a normal comparison should an epizootic occur. Arthropod and mollusc specimens are best preserved in 70% ethanol for most studies; 10% buffered formalin is not recommended in most terrestrial invertebrates, especially if preserving specimens intact. Formalin appears to penetrate cuticle and membranes poorly compared with ethanol. Cuticular hardening also makes histologic sectioning extremely difficult, and sections tend to fragment easily, limiting their diagnostic value.^{40,46}

Experience has shown that a variety of alcohols, including isopropyl and even vodka, act as better histologic fixatives for whole arthropod specimens than 10% buffered formalin.^{31,34}

Alternatively, specimens may be dissected in physiologic saline and tissues, then preserved in 10% buffered formalin, as often performed in entomology laboratories.¹⁹ This may still cause sectioning difficulties in species that have cuticular sections in the digestive tract, such as the sucking stomach in spiders¹¹ and the proventricular denticles of *Acheta* and *Gryllus* spp.

crickets. If preserving large terrestrial arthropods such as jungle nymphs (*Heteropteryx dilatata*) or large beetles (Orthoptera) intact, the cuticle should be incised to ensure adequate penetration of fixative, or fixative may be injected into the hemocoel cavity. I prefer staining with hematoxylin and eosin (H&E), as well as a trichrome stain (Masson, Martius scarlet blue), for all routine histology. Other useful stains include a Masson-Fontana or melanin bleach for evaluation of melanotic nodular-type reactions, a common response to cuticular injuries and some infections, in many arthropods. Gram stains have rarely been of any additional use. Most entomopathogenic fungi are clearly evident without the need for periodic acid-Schiff (PAS)-stained sections. Acid-fast-stained sections may be useful for demonstrating microsporidians, such as *Steinhausia* in *Partula* snails.⁴⁸

Marine corals with their calcium carbonate exoskeletons and extremely delicate soft tissues may pose problems with histologic examination and require special techniques. A study evaluating fixative and decalcification solutions on two species of hard coral (*S. siderea* and *P. porites*) and one species of soft coral (*G. ventolina*), demonstrated poor results with 10% seawater formalin.⁴⁹

An aqueous neutral-buffered zinc formalin solution (Z-Fix, Anatech) was found to be the best overall fixative, when the concentrate was mixed with artificial or ambient filtered seawater in a 1:4 ratio. The specimens may remain in fixative indefinitely. A significant advantage of this fixative is the suitability of fixed tissue for later molecular diagnostic techniques. Bouin's fixative is an alternative, in which case fixation must be for only 6 hours, before rinsing in 70% ethanol repeatedly, with storage in ethanol. If rinsing is inadequate, the picric acid will destroy the delicate tissues over time. Modified Helly's solution is another alternative, requiring 16 to 20 hours' fixation time, followed by rinsing in seawater several times over 24 hours, then storage in 70% ethanol. Repeated changes of ethanol in the first few days are needed. If the fixative is not totally rinsed before immersion in alcohol, an insoluble precipitate will form.

Corals may also be enrobed in agar to prevent loss of friable tissues during processing and to support and maintain the spatial orientation of tissue.⁴⁹ Enrobed coral may then be easily cut after decalcification and before processing to allow a specific orientation in the paraffin block. The agar will not penetrate coral exoskeleton, resulting in a void once the exoskeleton is removed by decalcification. Decalcification to remove the calcium carbonate exoskeleton or spicules in soft corals, or *gorgonians*, for histology processing is best performed with a solution of one of the sodium salts of

ethylenediaminetetraacetic acid (EDTA). A neutral solution will also allow the use of immunohistochemical staining or molecular techniques such as polymerase chain reaction (PCR). The time needed for complete decalcification varies. *Diploria* and *Isophyllastrea* have denser exoskeletons and take longer to decalcify than *Acropora*, with its porous exoskeletal structure.⁴⁹

Transmission and scanning electron microscopy (EM) samples for terrestrial and aquatic invertebrates are best preserved in glutaraldehyde and osmium tetroxide. Ideally for EM, moribund or anesthetized specimens should be dissected in glutaraldehyde, with postfixation in osmium tetroxide.¹⁹

If DNA studies or molecular techniques (e.g., PCR) are anticipated, freeze drying, storage in liquid nitrogen, or rapid freezing and storage may also be considered.

TARGETED DISEASE MONITORING

Once the initial pathogen screening has been performed, monitoring may be more targeted for the detection of the specific infectious agents deemed important. Simple light microscopic examination has been effective in the pre-release screening of captive-bred British field crickets (*Gryllus campestris*) for cephaline eugregarine parasites. This is another case in which initial screening unfortunately was not performed, and management decisions have been based on the assumption that infection is not present in the wild population. Light microscopy in this case is more rapid and cost-effective than histologic examination, while providing comparative accuracy. Representative samples of the population to be released are euthanized and the digestive tract removed and examined pressed between two glass slides. The large, Apicomplexa trophozoites are easily visualized if present.^{36,37} Examination of wet mounts or Giemsa-stained dry smears is also useful for diagnosing other protozoal infections.¹⁹

Simple light microscopy may in cases be sufficient for virology, once initial work has identified the viral taxa involved. Occluded viruses, such as nuclear polyhedrosis viruses (NPVs), granulosis viruses (GVs) belonging to the Baculoviridae, and cytoplasmic polyhedrosis viruses (CPVs) belonging to the Reoviridae, as well as Entomopoxviridae, may produce large, proteinaceous inclusion bodies, easily visible with simple differential staining such as Giemsa or Buffalo Black 12B.¹⁹ Iridoviruses and entomopathogenic bacteria may cause distinctive macroscopic color changes, clearly evident in diseased or dead specimens. Some viral diseases show distinct lesions in organs such as the

adipose bodies, also evident on light microscopy.⁵⁵ However, viruses such as the Picornavirus causing cricket paralysis are nonoccluded and will only be detectable on EM.¹⁹

Entomopathogenic fungi are usually easy to differentiate from opportunistic saprophytes, which may grow on the cuticle, especially with poor enclosure hygiene. Entomopathogenic fungi will invade hemolymph or organs, only forming externally visible fungal growth and spores once the animal is dead. Death is either by extensive fungal invasion of the coelom or by toxin production. In contrast, live animals with cotton-wool-like fungal growth macroscopically visible on the cuticle are usually simply affected by saprophytes, and topical treatments (e.g., iodine-based solutions) and change to a clean environment normally result in cure.

Metarhizium anisopliae fungal infections in endangered Frégate Island giant tenebrionid beetles (*P. herculeanus*) may be evident postmortem by small quantities of dirty-white fungal growth at the mouth. On opening the carcass, the adipose bodies are most often affected, being firm, white, and chalky in consistency. Fungal elements distinctive for *Metarhizium* are generally easily visualized on light microscopic examination of wet mount preparations of the material (Figure 11-1).

When screening moribund tarantulas for Panagrolaimidae nematode infection, care should be taken because of the possibly zoonotic nature of this infection.^{40,41,44} A slender pipette is used to flush the mouth with physiologic saline when screening live speci-

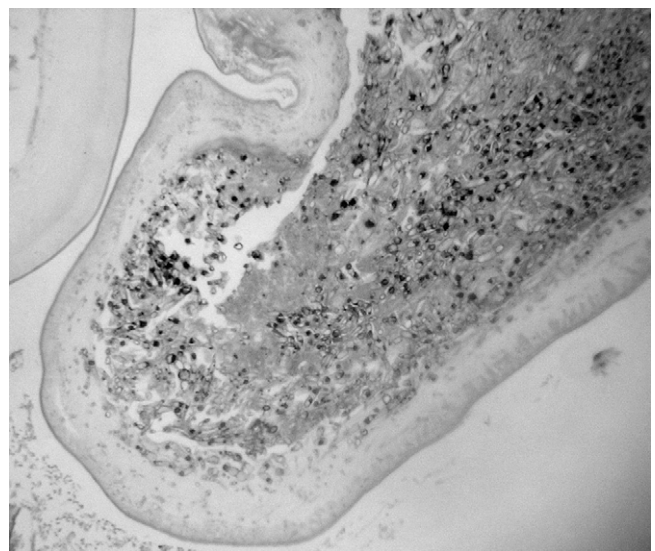


Fig 11-1 Melanization (nodule) reaction in response to a systemic *Metarhizium anisopliae* infection in larvae of the Frégate Island beetle (*Polposipus herculeanus*). (See Color Plate 11-1.)

mens. This may be facilitated by gas anesthesia of affected spiders.^{28,29,46} Immediate examination by low-power light microscopy readily demonstrates the small, motile rhabditid nematodes, if present.

When nematodes are to be preserved for later identification, this is best accomplished by slow killing to prevent curling, which makes identification difficult. Placing nematodes in 60°C water or saline for at least 2 minutes, followed by emersion in 4% buffered formalin, works well. Alternatively, Lacey¹⁹ reports that 4% buffered formalin may be gradually added to the saline containing the nematodes over several minutes. After several hours the nematodes should be placed in fresh 4% buffered formalin. Alcohols tend to result in distorted and brittle specimens.

Management of conservation insect populations infected with gregarines that may be destined for later release or reintroduction may be attempted by culling adults and sterilizing enclosures with 1:10 dilution of sodium hypochlorite (bleach). Rearing a new, disease-free culture may then be done from eggs and, for ease of monitoring, in small groups to allow representative culling of a small portion of each group for continued surveillance. If a group is found to be positive again, the whole adult population does not need to be culled, only the affected group. If this initial management is not sufficient, eggs may be briefly washed with an extremely dilute bleach solution (at least 1:100) for about 1 second to remove surface oocysts. This technique needs to be tried on a small number of eggs first because eggs are easily destroyed. It is also important to help prevent oocyst buildup by removing feces as regularly as feasible and limiting excess substrate, although this will not be possible in many management systems. These techniques are not ideally suited to a zoo exhibit enclosure and are easier to implement in nonexhibit insect groups. Adults submitted for surveillance postmortem examinations should be submitted for histology as well as fresh dissection because both techniques may occasionally miss gregarine infection (Figures 11-2 and 11-3).

Cases of intoxication are often difficult to diagnose definitively but have occurred in zoologic collections.⁵⁰ Distinctive evidence includes neurologic signs (e.g., uncontrolled twitching) and rapid death, which tend to affect large numbers or a large proportion of an enclosure population simultaneously. Although some toxins may cause tissue changes visible on histology, many do not. Pesticides used in adjacent buildings, as well as treated food plants, are common sources.³⁰ Another increasingly common problem appears to involve Fipronil, which may be transferred from the hands of staff who have handled other animals, due to

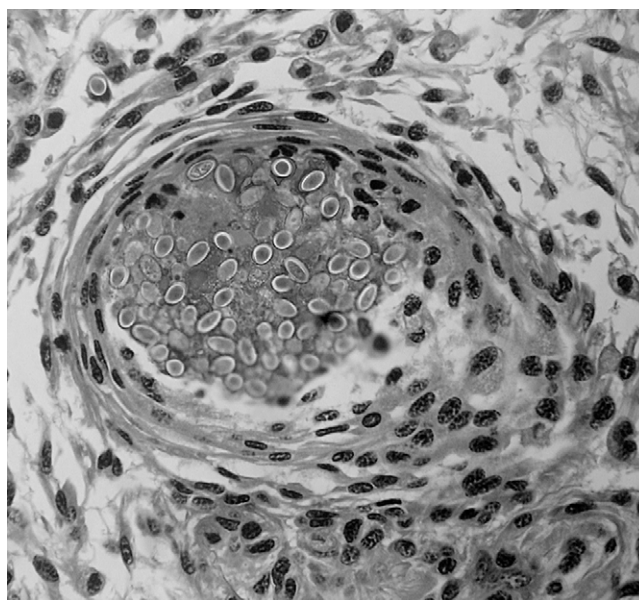


Fig 11-2 Melanized coelomic nodule containing gregarine parasite oocysts in a zoo-bred British field cricket (*Gryllus campestris*). This chronic infection was not detectable on fecal flotation. (Hematoxylin and eosin [H&E] stain.) (See Color Plate 11-2.)



Fig 11-3 Cephaline (septate) eugregarine from the gut of a desert locust (*Schistocerca gregaria*) in a zoologic exhibit. Oocysts were easily detectable from fecal flotation in this case. (H&E stain.) (See Color Plate 11-3.)

the residual nature of the compound. In one collection, despite repeated and attentive cleaning and scrubbing, enclosures that had held snakes treated for mites still killed all tarantula spiders placed in them for several months.⁴⁰

Examination of dead invertebrate specimens unfortunately is rarely useful. Some diseases (e.g., *Metarhizium* fungal infections) may be detectable for days after death, but other infectious agents are obscured within

a short time after death by the postmortem invasion of gut flora. When performing initial screening programs or investigating epizootics, it is essential that live, preferably moribund, specimens are selected. Post-mortem invasion is extremely rapid in invertebrates, easily confusing diagnosis of the cause of disease. Specimens should not be removed from their usual husbandry conditions until immediately before sampling. Relatively minor changes in temperature and humidity may cause rapid overgrowth of secondary invaders antemortem in moribund invertebrates,¹⁹ which needs to be taken into account.

ZOOLOGIC EPIDEMIOLOGY AND POPULATION MEDICINE

It is essential to remember that it is the *population* and not the individual animals that are of importance in invertebrate conservation programs. Therefore the approach to disease management is closer to that of production animal herd health than that of many other zoo animals managed as individuals.

In terms of population health, the shorter generation intervals and much greater population numbers of captive invertebrates allow the implementation of detailed epidemiologic investigations of mortality rates and fecundity. The extensive data on mortality rates in the different life stages of the *Partula* Polynesian tree snails at U.K. zoos are being correlated at the Zoological Society of London with changes in environmental conditions, as well as to decide on the most suitable archived material for further pathologic examination.

Regardless of how well intentioned, it is neither practical nor desirable for a veterinary pathologist to perform postmortem examinations on all invertebrate mortalities. More than 400 gross postmortem, bacteriologic, and cytologic examinations performed on *Partula* spp. of Polynesian tree snail over 5 years failed to yield a diagnosis as to cause of death.

Time may be better spent in analysis of population trends. Few zoo veterinarians embrace epidemiology, but in contrast to small captive populations of vertebrates, this is essential for invertebrates if the importance of a presumed disease etiology is to be evaluated. Population declines may be multifactorial, and environmental effects must also be considered.

An obvious exception to population management would be during the initial establishment of a small group of a rare species, especially females. The most notable example is the Lord Howe Island stick insect (*Dryococelus montrouzieri*). This species, perhaps the

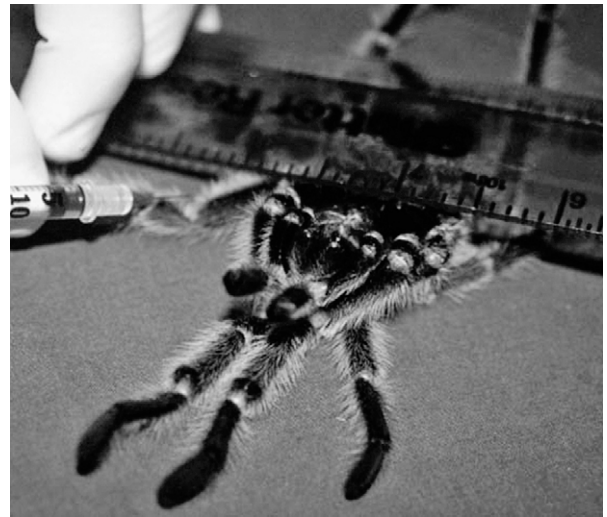


Fig 11-4 Sampling hemolymph from distal leg joint of tarantula for bacterial culture. Although the heart is easily accessible on the dorsum of the opisthosoma, contamination from the numerous gastric diverticuli makes this site unsuitable for culture. (See Color Plate 11-4.)

most endangered invertebrate species in the wild, was long believed extinct after the introduction of rats to the island. A tiny population of 27 individuals was found on Ball's pyramid, a large rock in the sea, 27 miles from Lord Howe Island. Two pairs were collected and sent to different zoos. The founder animals were irreplaceable, and an ailing founder female needed veterinary attention after laying her first batch of eggs at the Melbourne Zoo.

A growing body of literature is addressing clinical veterinary techniques for other, large, charismatic invertebrates such as tarantulas, which may live more than 20 years^{3,12,20} (Figure 11-4). This includes surgical techniques,^{32,33,40,42,43} fluid administration,^{39,40} and therapeutics.⁶¹ Individual animals are generally of limited value in most invertebrate conservation programs and thus are not covered further here.

CONCLUSION

Increasing numbers of invertebrate taxa are being kept in zoologic collections, so it is essential that disease-screening protocols are instituted when captive colonies are first established, particularly in species for which possible future release programs are to be considered. Consistent protocols are needed among all involved collections. Veterinarians, pathologists, and invertebrate curators need to rise to the challenge to limit the risk of introducing pathogens into naive endangered invertebrate populations in their natural habitat.

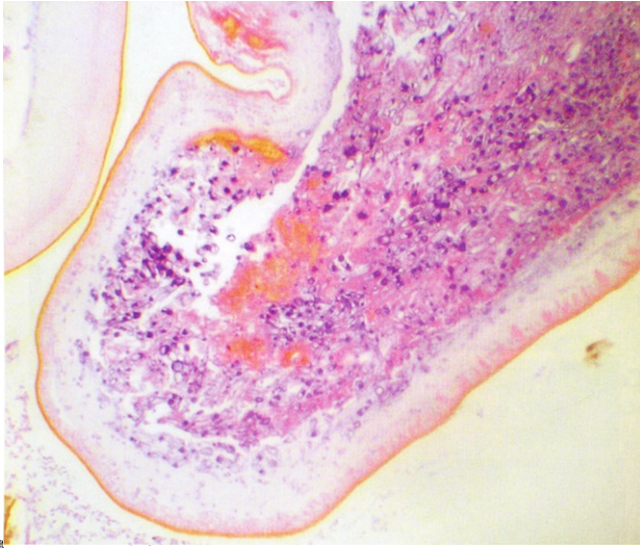
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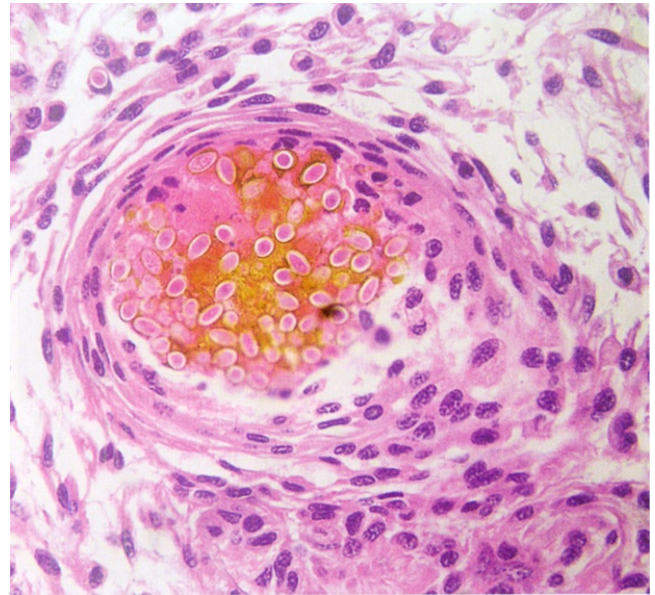
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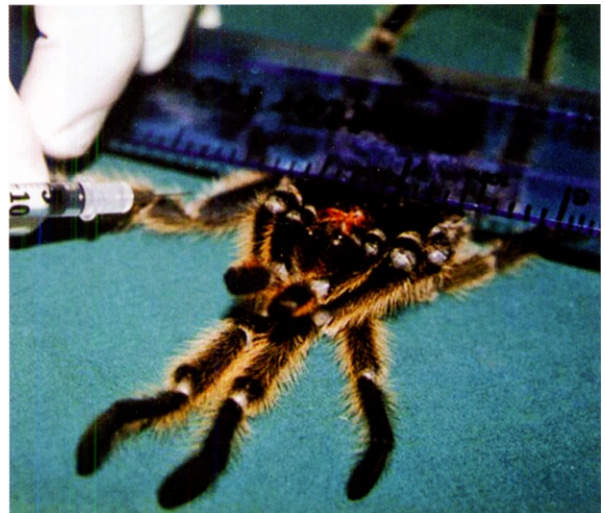
Color Plate 11-1 Melanization (nodule) reaction in response to a systemic *Metarhizium anisopliae* infection in larvae of the Frigate Island beetle (*Polposipus herculeanus*). (For text mention, see Chapter 11, p. 92.)



Color Plate 11-2 Melanized coelomic nodule containing gregarine parasite oocysts in a zoo-bred British field cricket (*Gryllus tempestris*). This chronic infection was not detectable on fecal flotation. (Hematoxylin and eosin [H&E] stain.) (For text mention, see Chapter 11, p. 93.)



Color Plate 11-3 Cephaline (septate) eugregarine from the gut of a desert locust (*Schistocerca gregaria*) in a zoologic exhibit. Oocysts were easily detectable from fecal flotation in this case. (H&E stain.) (For text mention, see Chapter 11, p. 93.)



Color Plate 11-4 Sampling hemolymph from distal leg joint of tarantula for bacterial culture. Although the heart is easily accessible on the dorsum of the opisthosoma, contamination from the numerous gastric diverticuli makes this site unsuitable for culture. (For text mention, see Chapter 11, p. 94.)

CHAPTER 12

Use of Wildlife Rehabilitation Centers as Monitors of Ecosystem Health

JONATHAN M. SLEEMAN

Wildlife rehabilitation is defined as the temporary care of injured, diseased, and displaced indigenous animals and the subsequent release of healthy animals to appropriate habitat in the wild.³⁷ However, this definition does not include the role of euthanasia as an appropriate disposition for many wild animals brought to wildlife rehabilitators. Few have doubted the important role of these activities in improving the welfare of many wild animals that are often injured as a result of human activities. However, it is unlikely that the rehabilitation of injured individuals of a common species has any significant effect at the population level.⁵⁵ Therefore, legitimate questions have been raised regarding the justification for such activities and whether they could lead to the interference in natural selective processes, increase disease transmission among and between species, and result in the inappropriate translocation of animals. The limitations of wildlife rehabilitation as a conservation tool are discussed elsewhere.^{3,29}

It is important that policies and practices are established that eliminate or minimize the potential harm that could result from wildlife rehabilitation, as well as expand on the traditional role of the treatment and release of wildlife to include many other educational, research, conservation, and public policy initiatives.⁴⁸ This chapter provides an overview of how wildlife rehabilitation centers may contribute to the monitoring of ecosystem health, which is defined as the maintenance of biodiversity and ecosystem integrity. Specifically, this discussion outlines the elements of a preventive medicine program designed to minimize the potential harm from wildlife rehabilitation. Furthermore, the concept of conservation medicine as it relates to wildlife rehabilitation is introduced, and two examples are used to illustrate how data from a wildlife rehabilitation center have been used to monitor ecosystem health.

PRIMUM NON NOCERE (FIRST DO NO HARM)

One of the guiding principles of veterinary medicine is “first, do no harm.” The welfare of wildlife populations is more important than the welfare of any individual animal, and nothing done in the interest of an individual animal should unnaturally jeopardize healthy wildlife.⁵² In this context, it is important to have a comprehensive preventive medicine program designed for the specific geographic location and circumstances to prevent harm to human health, individual animals, the wildlife populations, and the environment as a result of wildlife rehabilitation activities. This program should consist of admittance and release policies, animal identification and medical record systems, feeding and nutrition protocols, appropriate caging design and handling protocols to minimize trauma, standard sanitary operating procedures, wildlife health screening protocols, quarantine and isolation, and a staff occupational health program.

The protection of public health is paramount to all activities at a wildlife rehabilitation center. Therefore, it must be determined whether acceptance of any species represents an unacceptable risk to human health. For example, the recovery and admission for treatment of adult rabies-vector species, such as striped skunks (*Mephitis mephitis*) and raccoons (*Procyon lotor*), may present an unacceptable threat of exposure to rabies virus both to the general public who may encounter these animals and to the staff of the rehabilitation center.⁴⁶ The risk of injury from venomous species as well as physically dangerous animals, such as adult white-tailed deer (*Odocoileus virginianus*), should also be assessed before allowing the admission of these species.

To prevent disease transmission within a wildlife center (which has been documented on several

occasions^{18,22}), strict hygiene and sanitation should be maintained that includes standard sanitary operating procedures¹⁰ as well as pest and rodent control. In addition, all animals that are admitted should undergo a comprehensive health screening examination that varies between species to reflect different disease risks. The tests performed and prophylactic treatments administered could be based, for example, on the World Organization for Animal Health (OIE, Office International des Epizooties) publication *Quarantine and Health Screening Protocols for Wildlife prior to Translocation and Release into the Wild*.⁵⁶ Other examples of a preventive medicine program include the isolation of any sick animal with a communicable disease from healthy animals, as well as the quarantine of healthy animals suspected of carrying an infectious disease before disease screening. These animals should be housed in purpose-designed quarantine rooms that include separate airflow and drainage and separate caging and equipment from the rest of the building. An occupational health program for the staff could include annual rabies titers and vaccination if necessary, policies on access to rabies-vector species and dangerous animals, dissemination of information on wildlife zoonoses, and annual educational programs on health and safety, including classes on appropriate restraint and handling techniques.

Finally, the release policies should be designed to minimize the potential for disease introduction into the wild and for harm to the wild populations and the environment. The animal must not pose a risk to the wild population, humans, or the environment and should not be likely to spread pathogens or contribute to disease processes in other ways. The animal must not be carrying a potentially zoonotic infection and should not be imprinted, tame, or habituated to humans. In addition, animals must be released at, or near, the original site of capture unless conservation efforts or safety considerations dictate otherwise. The habitat must be within carrying capacity for the species to be released.

Prevention of interference in natural selection is probably more difficult to achieve. However, few (if any) wildlife populations in heavily altered ecosystems, such as exist in many parts of North America, escape anthropogenic effects on their health. In fact, recent reviews of the morbidity and mortality of wildlife presented to universities and wildlife centers have identified that human activities account for the majority of admissions.^{5,15,53} Even less obvious reasons for admission, such as aural abscesses in box turtles (*Terrapene carolina*) and *Eustrongylides ignotus* infection in herons and egrets, may be the result of human activ-

ities, such as contamination of the environment with organochlorine pesticides²⁵ and eutrophication of wetland habitats,¹⁴ respectively. In contrast, wildlife from more pristine ecosystems may be under less anthropogenic influence, and consequently more consideration must be given to the potential deleterious effects of rehabilitation on the genetics of the population. Although no systematic studies have been conducted, some apparently pristine areas are also likely under human pressures, although somewhat different from those encountered in developed countries. For example, the illegal wildlife trade accounts for the majority of wildlife confiscations that are admitted to zoos and wildlife rehabilitation centers in Central and South America.⁴⁰

Some species are afforded more legal protection, that is, threatened and endangered species, whereas others are classified as “nuisance” or “invasive” species. Also, most wildlife rehabilitation centers have limited resources. Thus, a triage policy should exist such that more time and resources are allocated to the animals from populations that would benefit most from these efforts, such as threatened and endangered species. It is also essential that wildlife rehabilitation centers comply with all state and federal laws, including those that may prohibit the release of certain nuisance species, for example, coyotes (*Canis latrans*) in Virginia.⁵² In addition, some introduced species, such as starlings (*Sturnus vulgaris*) and mute swans (*Cygnus olor*), are known to compete successfully with native species for nesting sites.⁴¹ Consequently, I believe that nonnative, invasive, and nuisance species should not be rehabilitated or released into the wild.

CONSERVATION MEDICINE

The importance of veterinary medicine and environmental education as part of a multidisciplinary approach to wildlife conservation and ecosystem health has previously been identified.¹ *Conservation medicine* is a new discipline and has developed in response to the emergence of new diseases and threats to human and animal health from anthropogenic ecologic changes. Conservation medicine examines ecologic health issues, including emerging diseases; the biologic effects of pollutants; the health implications of ecologic alterations such as habitat fragmentation, simplification, and degradation; loss of biodiversity and ecosystem services; and global climate change. Conservation medicine is by definition transdisciplinary, involving professionals from diverse disciplines, such as human

medicine, public health and epidemiology, veterinary medicine, ecology, conservation biology, wildlife management, and the social and political sciences, as well as educators and policy makers.

Wildlife rehabilitation centers are in a unique position to observe these ecologic changes and anthropogenic effects on wildlife health. Therefore, wildlife species presented for treatment are potentially excellent biomonitors for environmental problems. Many species, especially raptors, amphibians, and marine mammals, have provided information about environmental pollution and emerging diseases.^{13,17,23,42,47} Reptiles may also be good indicators of ecosystem health because of their reliance on the environment for appropriate physiologic functions, such as immunity, digestion, and thermoregulation.⁴⁵ Data from wildlife rehabilitation centers have been underutilized for this purpose. However, the following studies demonstrate how data collected at a wildlife rehabilitation center may be used to monitor ecosystem integrity and the maintenance of biodiversity. These studies also illustrate the usefulness of wildlife as sentinels for human health threats.¹⁷

Aural Abscesses in Box Turtles as an Indication of Environmental Contamination

Aural abscess represented the second most common diagnostic category in free-living eastern box turtles (*Terrapene carolina carolina*) admitted to the Wildlife Center of Virginia (WCV, Waynesboro) from 1991 to 2000⁵ and has been reported in other states.⁵⁴ Aural abscesses are accumulations of caseous debris in the tympanic cavity resulting from squamous metaplasia of the epithelium that lines the middle ear.^{7,25} The lesion, which may be unilateral or bilateral, varies in size from subclinical accumulations of material to masses large enough to impede the animal from drawing its head back into the shell (Figure 12-1). Aural abscesses in free-living eastern box turtles resemble lesions occurring in captive box turtles that are vitamin A deficient.³⁹ A similar relationship between hypovitaminosis A and squamous metaplasia has been described in other species, including ruminants and pigs.^{8,19}

High body burdens of organochlorine (OC) compounds were associated with aural abscesses and squamous metaplasia of mucin-secreting epithelial tissues of the upper and lower respiratory passages, eyes, and the middle ear in free-living eastern box turtles.²⁵ It was hypothesized that increased levels of OC compounds disrupted vitamin A metabolism, leading to



Fig 12-1 Bilateral aural abscesses in an eastern box turtle (*Terrapene carolina carolina*).

hypovitaminosis A, squamous metaplasia, and accumulation of caseous debris in the middle ear of these turtles, visible grossly as aural abscesses. This is consistent with previous studies on humans, rats, and turtles that showed that increased exposure to OC compounds led to a disturbance in vitamin A homeostasis^{11,44} and to upper respiratory infections.⁵¹

A study of the epidemiologic determinants of aural abscesses in free-living eastern box turtles in Virginia found that affected turtles were geographically clustered (Figure 12-2).⁶ In fact, geographic location was the only risk factor associated with aural abscesses. Thus the geographic location of this condition in free-ranging eastern box turtles may be useful as an indicator for the presence of OC compounds in the environment, if a causal relationship between body burdens of OC compounds and aural abscesses in box turtles can be determined. Box turtles may serve as good indicators of environmental contamination because they are abundant, have a wide distribution with a small home range, have an intricate relationship with the environment, and are long lived.^{21,24,38,43,45}

OC compounds are also hypothesized to be, or have been, causally linked to a number of diseases in humans, including but not limited to chloracne,¹¹ neurologic dysfunction,²⁸ breast and other cancers,^{9,26} hypospadias and other birth defects,⁴ and endometriosis.³³ It would be of particular interest to determine if these OC-associated diseases in humans were clustered in the same geographic regions as box turtles with ear abscesses. This would illustrate the link between human and wildlife health, as well as the sentinel function of wildlife.

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Fig 12-2 Numbers of eastern box turtles (*Terrapene carolina carolina*) submitted to a wildlife rehabilitation center from counties of Virginia, 1991–2000. Counties from which eastern box turtles were received are shaded gray (dark gray for counties with aural abscess cases, light gray for counties from which none of the submitted turtles had aural abscesses). Pie charts in counties with aural abscess cases indicate county proportion of turtles with aural abscesses in black, and turtles with no aural abscess in white. (From Brown JD, Sleeman JM, Elnger F: J Wildl Dis 40:704-712, 2004.)

Impact of West Nile Virus on Raptor Populations

West Nile virus (WNV) is a widely distributed arbovirus of the family Flaviviridae. Birds are the principal vertebrate host,³¹ and historically, clinical disease is rare in birds.⁵⁰ WNV was first detected in North America during an outbreak of viral encephalitis in New York in 1999.⁵⁰ Since its initial detection in the Western Hemisphere, this emerging infectious disease has rapidly spread across North America, resulting in morbidity and mortality of humans and a broad range of vertebrate species.³⁶ North American avian species appear to be particularly susceptible to the disease, and more than 230 avian species are listed in the Centers for Disease Control and Prevention (CDC) West Nile virus avian mortality database,* of which 15% (35/234) are native North American raptor species. Several reports describe mortality of raptors resulting from WNV infection.^{12,16,32,34} In addition, recent authors have speculated that WNV has resulted in regionally increased raptor mortality in the United States.^{16,35} The impact of WNV on avian populations is unknown, but some have predicted a likely negative effect in the forthcoming years.²

A recent study investigated some epidemiologic findings of a WNV outbreak in raptors from Virginia

during 2003 by analyzing changes in trends of admissions to a wildlife rehabilitation center for various species of raptors.²⁷ Annual juvenile raptor admissions from 1993 to 2003 for all species, great horned owls (*Bubo virginianus*), and red-tailed hawks (*Buteo jamaicensis*) were calculated and graphically plotted. The mean monthly number of admissions for all raptor species, great horned owls, and red-tailed hawks admitted between 1993 and 2002 was also plotted. Finally, the monthly admissions for all raptors, great horned owls, and red-tailed hawks during 2003 were plotted and compared to previous 10-year mean monthly admissions.

The 2003 raptor admissions showed a decrease during May and June, speculated to be caused by nestling mortality from WNV infection, compared with the mean of the previous 10 years. In addition, a similar comparison with historical data showed increased admissions during August and September in 2003 resulting from cases of WNV infection, followed by a decrease from October to December, thought to result from the decline in fledgling birds normally presented at that time of year. Red-tailed hawk admissions also showed a mild increase during June and August, with a marked increase in September 2003 (Figure 12-3). This was followed by a mild decrease in red-tailed hawk admissions during October and November and a marked decrease in December. In addition, there was a marked decrease in the number of juvenile great horned owl admissions during 2002 and 2003 compared with the annual admissions from 1993 to 2001,

*<http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm>.

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Fig 12-3 Comparison of monthly admissions of red-tailed hawks (*Buteo jamaicensis*) to the Wildlife Center of Virginia during 2003 and the mean monthly admissions plus one standard deviation (+1 SD) for red tailed hawks, 1993–2002. (From Joyner PH, Kelly S, Shreve AA, et al: J Wildl Dis 42:335-344, 2006.)

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Fig 12-4 Annual distribution of immature great horned owls (*Bubo virginianus*) admitted to the Wildlife Center of Virginia, 1993–2003. (From Joyner PH, Kelly S, Shreve AA, et al: J Wildl Dis 42:335-344, 2006.)

speculated to be caused by nestling mortality from WNV infection (Figure 12-4).

The change in the trend of monthly distribution of raptors admissions, as well as the decrease in annual immature raptor admissions (especially great horned owls), may indicate declines in local populations. Although many environmental factors may be responsible for potential population declines, the most plausible hypothesis was WNV having a negative impact on local raptor populations. This was supported by that fact that the species showing the most dramatic changes in admissions, red-tailed hawks and great

horned owls, accounted for the majority of WNV cases (78% of all cases). Although WNV in raptors could not be proved as the cause of these changes in trends, this study illustrates the usefulness of monitoring trends in admission data to generate hypotheses about the impact of disease on wildlife populations. A decrease in raptor populations would again have public health implications. Raptors are ecologic keystone species and important regulators of rodent populations. Loss of this regulator would result in increased rodent populations and possibly increased rodent-associated zoonotic infections.

EDUCATION, PUBLIC AWARENESS, AND PUBLIC POLICY

Members of the public generally have a concern for wildlife and the environment. Wildlife rehabilitation centers may use clinical cases and experiences to educate the public about the value of wildlife and the importance of healthy ecosystems. Ultimately, conservation of wildlife and the environment requires the support of the public, appropriate protective legislation, and adequate law enforcement. Clinical work and research conducted at wildlife rehabilitation centers should help to influence conservation-related public policy decisions.

Education of the general public is also important to promote the prevention of many human-induced diseases. For example, one study demonstrated significant temporal changes in reptile morbidity and mortality, especially road mortality, both in absolute number and in proportion to the total caseload of animals presented to a wildlife rehabilitation center⁵ (Figure 12-5). This study showed two peaks of mortality: the first in May and June, indicative of the natural period of breeding and nesting, and the second in September, speculated to represent increased activity before hibernation. There is also evidence that the current prevalence of road mortality in the eastern and central United States may jeopardize population persistence of land turtles and large-bodied pond turtles.²⁰ Therefore, it is important to use these types of data to educate people about wildlife health issues and

give advice about how to avoid injury to animals and, in this example, prevent further road mortality. These data are also important to help target education campaigns at peak periods of mortality.

Many wildlife rehabilitation centers also have programs for veterinary students and recent graduates. Ecosystem health should be incorporated into the curriculum. The students should be encouraged to consider the “bigger picture” and expand on the traditional physician-patient paradigm to look at regional and global ecologic changes that may be influencing diseases in their patients. This creates a more preventive medicine-oriented approach in their thinking, fosters an appreciation for biodiversity, enhances social responsibility, and produces advocates for a healthy environment.⁴⁸

CONCLUSION

Wildlife rehabilitation centers may contribute to a wide variety of important activities that may not only enhance animal welfare, but also advance veterinary science and veterinary education, biodiversity conservation and ecosystem health, wildlife health monitoring, public health, and biosecurity, as well as public education and conservation-related public policy. For these roles to be realized, more emphasis should be placed on accurate and detailed medical record keeping, standardized health screening, and more thorough clinical examinations, ancillary diagnostic

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Fig 12-5 Monthly distribution of reptile cases presented to the Wildlife Center of Virginia, 1991–2000. (From Brown JD, Sleeman JM: *J Wildl Dis* 38:699-705, 2002.)

tests, and postmortem examinations on animals that die or are euthanized. In addition, it is important to realize the limitations of the data as well as potential biases, because admissions to wildlife rehabilitation centers are also certainly biased toward human-induced diseases.^{30,49}

Acknowledgments

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CHAPTER 13

Biopsy Darting

WILLIAM B. KARESH

REMOTE BIOPSY TECHNIQUES

The widespread use of biopsy punches adapted for remote sample collection began in the 1980s.^{1,11} The technique allows for the collection of biologic materials without the need for, and the risks associated with, the capture and handling of animals. Before techniques for amplifying genetic material, such as polymerase chain reaction (PCR), were developed, biopsy samples were used in cell cultures to produce fibroblast cell lines and provide adequate amounts of deoxyribonucleic acid (DNA) for genetic analysis.

Subsequently, molecular genetic techniques advanced sufficiently to allow analysis without the need for cell culture. These same techniques have permitted genetic analyses to use other, noninvasively collected samples, such as feces, hair, or shed epithelial cells, and have led to some innovative approaches, such as netting sloughed skin from the surface of water around breaching whales or putting duct tape on the tip of a plastic syringe dart to pluck hair samples from primates.^{5,13}

The ability to perform genetic analyses directly on biopsy material rather than requiring successful cell culture has also increased the utility of biopsy darting by eliminating the sample losses caused by bacterial and fungal contamination and making sample storage simpler. Concomitant with advances in genetic analytics, remote biopsy collection has become widely used when other options should not be employed. Increasingly, the technique is used to answer questions not related to genetics, such as those involving infectious and noninfectious diseases, toxicology, and biomarker assessment.^{4,6,7,15}

Biopsy darts have been used in a wide range of vertebrate species, including more than 30 species of cetaceans, as well as many species of pinnipeds, carnivores, primates, ungulates, and birds^{3,7-12} (Box 13-1). Darts have even carried by teams searching for “Sasquatch” in the United States. The most common delivery mechanism for the biopsy instrument uses a dart projector (e.g., pistol, rifle), a crossbow, or a

Box 13-1

Partial Listing of Taxonomically Grouped Species Reported to Have Been Sampled Using Biopsy Darting Techniques

American alligator	Orangutan
Ostrich	Lowland gorilla
Bottlenose dolphin	Patas monkey
Common dolphin	Domestic horse
Striped dolphin	Zebra
Killer whale	Prezwalski horse
Humpback whale	African elephant
Fin whale	<i>Rhinoceros</i> spp.
Right whale	Gaur
Gray seal	American bison
Southern elephant seal	Giraffe
Southern fur seal	Okapi
South American sea lion	Greater kudu
Walrus	Impala
Lion	Waterbuck
Sun bear	Lechwe
Brown bear	Kob
African wild dog	Puku
Spotted hyena	Bighorn sheep

mounting on the end of an extension pole (Figures 13-1 through 13-4).

BIOPSY DARTING EQUIPMENT

Following the early development and production of biopsy darts by individuals or teams of researchers in the 1980s, biopsy darting equipment has become commercially available from a number of manufacturers (Box 13-2). Most of the biopsy tips produced commercially are adapted to drug injection darting equipment.

All systems use the concept similar to a standard biopsy punch: a metal cylinder with a sharpened edge that cuts through the skin and underlying tissues. The tissue is held within the biopsy punch tip by friction



Fig 13-1 Darted impala. (See Color Plate 13-1.)



Fig 13-2 Darted zebra. (See Color Plate 13-2.)

or by barbs provided through the addition of either notches or protrusions from the inner wall of the punch cylinder, or by separate barbed hooks added inside the cylinder, such as the use of barbed broaches used in dentistry.

Figure 13-5 illustrates commercially available biopsy darts. The Telinject system (not illustrated) uses a cutting tip that fits one of the company's plastic syringe darts, combined with an insert piece that resembles three parallel-barbed dental broaches. The Palmer Cap-Chur biopsy tip screws on the company's

metal dart barrels and uses standard coarse-grade barbed dental broaches. It may be mounted on a variety of projector devices, with fittings available from the company. This product is also available with a notched indent on the side of the biopsy punch that allows air to escape and provides additional tissue-holding strength. The Pneu-Dart biopsy tip attaches to the front of one of the company's projectile syringes, with the injection needle modified to serve as the barbed tissue holder. The Dan-Inject biopsy tip is longer and thinner than the other commercially available

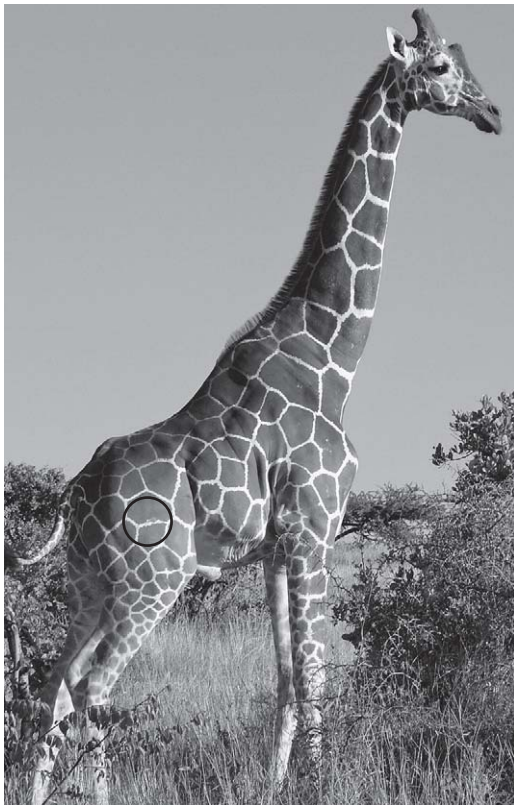


Fig 13-3 Giraffe darted in right hindquarter. (See Color Plate 13-3.)

Box 13-2

Commercial Producers of Biopsy Darting Equipment and Supplies

Palmer Cap-Chur Equipment Co., Inc. (www.palmercap-chur.com): syringe dart, crossbow bolt, arrow or extension pole

Dan-Inject ApS (www.dan-inject.com): syringe dart

Pneu-Dart, Inc. (www.pneudart.com): syringe dart

Telinject GmbH (www.telinject.de): syringe dart

systems and uses friction to retain the biopsy sample within the tip. It has a side-port opening at its base to allow air pressure to escape behind the tissue sample, thus helping to prevent the sample from falling out of the tip.

For all these systems, the biopsy cutting tip works best when it strikes a relatively firm body surface lying in a plane perpendicular to the dart. Soft or pliable target areas, such as body areas with loose skin or abundant subcutaneous fat, may disperse impact energy and prevent the biopsy dart from making a complete skin incision. Most darting systems may effectively penetrate thick hair coats. For very-thin-skinned or more delicate animals, a rubber plunger



Fig 13-4 Biopsy sample of same giraffe in Figure 13-3. (See Color Plate 13-4.)

from a 3-mL syringe may be slid down to the base of the biopsy tip to shorten the depth of penetration and serve as an impact absorber.

Unless the biopsy tip is attached to an extension pole or a retrieval cord, the projectile needs to recoil or bounce off the animal immediately, which is most common, or to fall out before losing sight of the darted individual. In thick-skinned animals such as elephants and rhinoceroses, the darts frequently stay in the skin until the animal's movement and gravity cause it to fall free. This could also be a result of the tendency to shoot long distances at large species; thus the dart hits with lower impact and less recoil energy to bounce out.

For aquatic use, the biopsy dart needs flotation material or a retrieval cord to prevent the loss of the darts and samples. Retrieval cords may also be useful in colony situations, where other group members would be disturbed if the researcher approached the animals to retrieve the dart from the ground.

Finding the biopsy dart once it has fallen off the animal may be quite challenging. In addition to

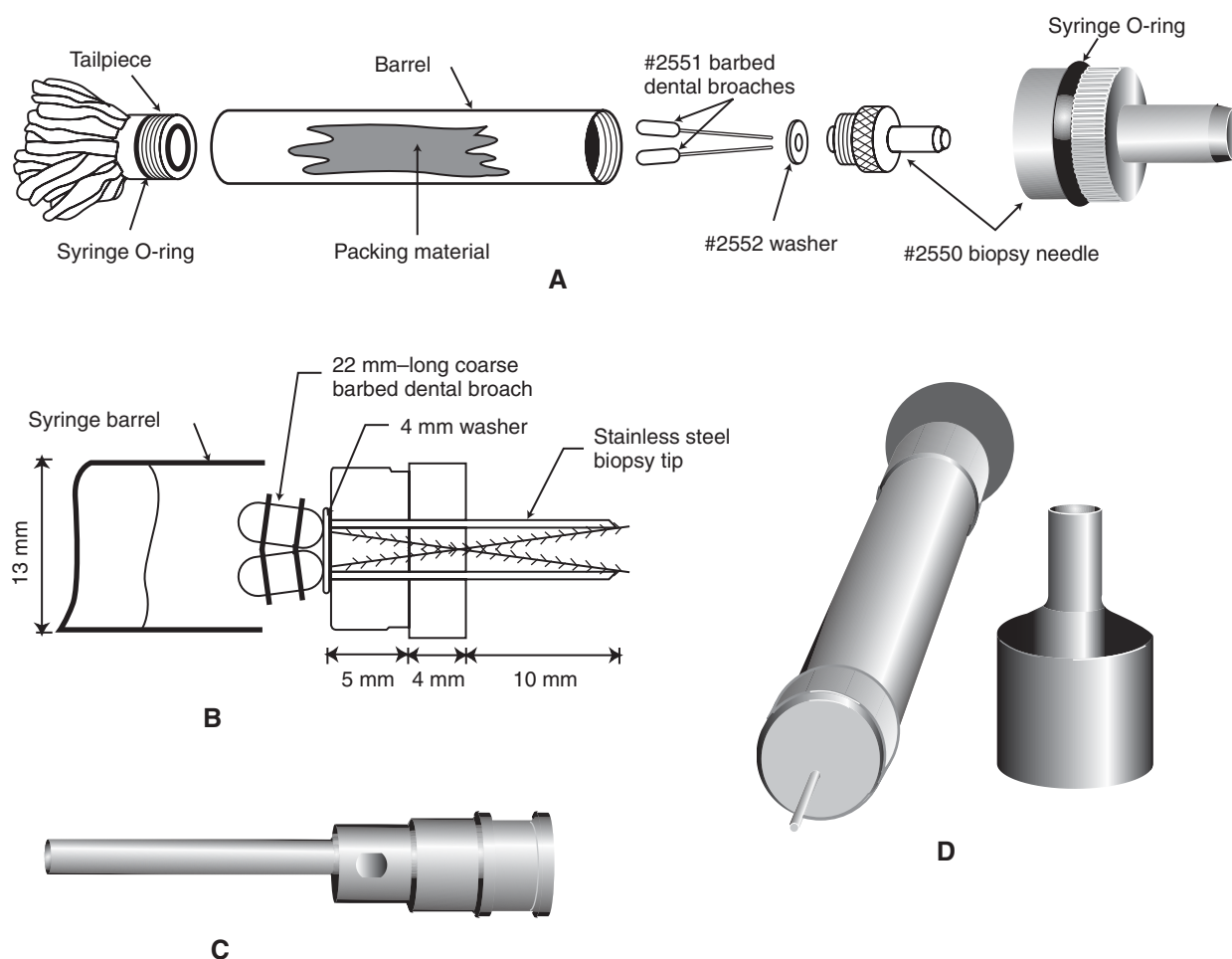


Fig 13-5 Commercially available biopsy darting systems. The Palmer Cap-Chur biopsy tip screws on the company's standard metal dart barrel (**A**) and uses standard coarse-grade barbed dental broaches (**B**). The Dan-Inject biopsy tip (**C**) fits on the company's projectile syringe and has an air exit hole at its base to prevent sample loss. The Pneu-Dart biopsy tip attaches to the front of one of the company's projectile syringes (**D**), with the injection needle modified to serve as the barbed tissue holder. (Images are not to scale.)

retrieval or tether cords, other methods include painting the dart a bright color, painting the dart with cold luminescent material and darting at dusk, using radio transmitter tailpieces for the darts, and using a metal detector. Dan-Inject also sells a dart syringe fitted with a passive diode for radio location by the Recco search system, providing a maximum search radius 15 to 20 m (50-66 ft).

DART SELECTION

Although reports of adverse reactions after biopsy darting are rare, researchers must always use the same level of caution required for any type of darting.^{2,14} To avoid traumatic injuries to the animal, appropriate matching of the size, weight, and impact of the dart to the physical characteristics of the animal is essential. The fact that immobilization agents or other drugs are

not being delivered in the darting system does not justify a lack of training and practice in darting procedures before working with live animals. Appropriate sterilization techniques are essential to prevent infectious agent transmission to or among animals being sampled. The discovery of prion-related diseases may pose additional considerations for sterilization techniques in the future.

Concern for behavioral disturbances must be applied, not only for the direct effect on the individual, but also for how the individual's reaction may affect others in the group, such as abandonment of young, displaced aggression, or stampeding. It must be recognized that dart tips, like injection needles, may break off in the animal, especially if they penetrate bone or other extremely hard tissues. As with any type of darting, the impact of overpowered darts may cause severe soft tissue damage, abdominal penetration, or skeletal fractures. Inaccurate darting or sudden movement of

animals may result in a dart hitting easily damaged body parts, such as eyes or genitalia.

Personnel also need to be properly trained to prevent darting-related accidents in other staff and to handle samples properly to avoid zoonotic infections.

When used correctly, biopsy darting remains a valuable tool for obtaining samples for a variety of diagnostic and research purposes. It also provides an alternative to the capture and handling of wildlife and to lethal sample collection methods.

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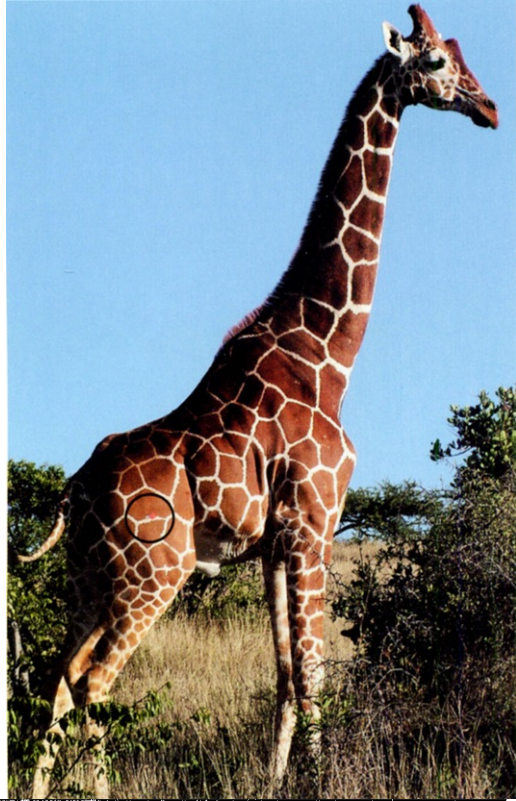
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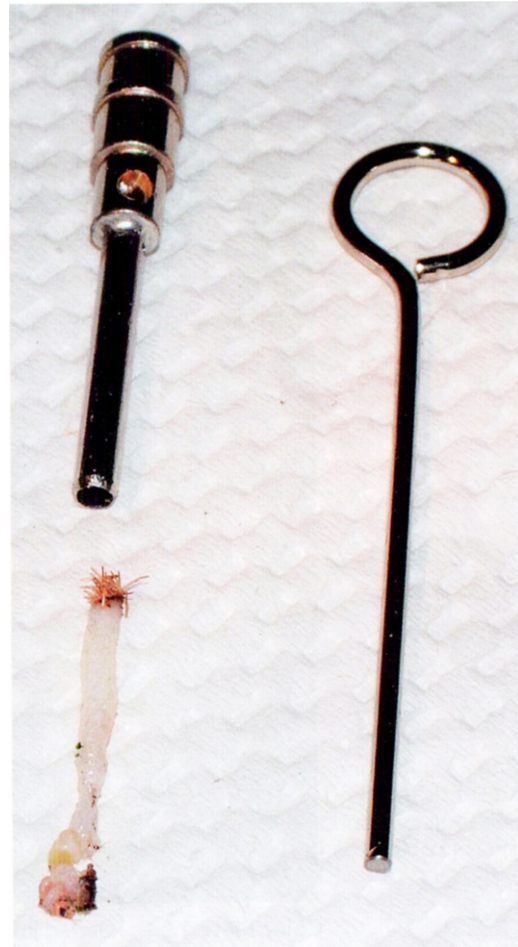
Color Plate 13-1 Darter impala. (For text mention, see Chapter 13, p. 106.)



Color Plate 13-2 Darter zebra. (For text mention, see Chapter 13, p. 106.)



Color Plate 13-3 Giraffe darted in right hindquarter. (For text mention, see Chapter 13, p. 107.)



Color Plate 13-4 Biopsy sample of same giraffe in Figure 13-3. (For text mention, see Chapter 13, p. 107.)

CHAPTER 14

Selected Fish Diseases in Wild Populations

FRANCIS T. SCULLION

Fishing is a vital source of income for local communities and a popular pastime in many countries of the world. An initial report of wild fish health problems usually originates from the fishing community. The majority of reports therefore concern fish of economic or sporting interest. The most obvious indicator of health difficulties in wild fish populations is mass mortality, but decreased numbers, abnormal behavior, or visible gross external lesions in wild fish are also reported.

The central tenet of disease investigation in wild fish is to establish a thorough systematic routine to collect and record evidence, in dramatically differing settings ranging from mountain streams to the open sea, that will eventually lead to a diagnosis. Establishing a disease diagnosis is strengthened by the supporting history, identification and prevalence of known pathogens or environmental factors that cause disease, associated clinical signs and pathology, investigator experience, and knowledge of the scientific literature.

UNDERSTANDING THE AQUATIC ENVIRONMENT AND ITS EFFECTS ON FISH HEALTH

Veterinarians understand the concept that disease agents are influenced by environmental factors. Within many animal production systems, however, the relevance of environmental effects is often alleviated by attention to husbandry, housing, nutrition, and other disease prevention techniques. In the wild, environmental factors influence the occurrence of disease unabated, and this concept must be further considered in any investigation of wild fish health problems.^{2,3}

Many natural processes affect the physical and biochemical parameters of rivers, lakes, estuaries, and oceans, and fluctuations may occur seasonally, diurnally, or tidally, depending on the parameters in question.

Box 14-1 lists the main parameters that vary naturally in aquatic systems and have a role in the overall health of animals living in this environment. Populations of wild fish live in equilibrium in the presence of many disease agents. Interactions and equilibria are a feature of the “big picture” within which the living animal strives to maintain homeostasis. Stressful changes in the environment allow pathogens to become established and to become detrimental to the health of the fish. Disease investigation involves the collation of information to ascertain which environmental and pathogenic elements have caused the problem.

DISEASE INVESTIGATION

In all situations, you should remember that the evidence and results of an investigation might be used in legal proceedings, so it is important to develop techniques that will withstand the judiciary process. This includes accurate sample identification, the collection of photographic data, and development and thorough recording of the chain of evidence.

History Collection

Record details of the person reporting the problem and ascertain the site of the problem accurately, with map references if possible. Some areas are remote, and because it is impossible to contain the site, changes often may occur before any investigation begins, and what is witnessed on one day may be different on another. Therefore you should obtain a good description of exactly what the initial reporter has seen. What is the obvious problem: dead fish, live fish swimming abnormally, lesions noted on live fish? When did the problem start? What type of fish are involved, and how many are affected? If the principal investigator

Box 14-1**Environmental Parameters That May Influence the Occurrence of Disease in Aquatic Systems**

Alkalinity
 Ammonia
 Color
 Dissolved organic and inorganic chemicals
 Dissolved oxygen concentration
 Nitrates
 Nitrites
 Particulate matter
 pH
 Salinity (marine environment)
 Temperature
 Turbidity
 Water hardness

cannot reach the site for some time, and if reporting personnel appear competent or are trained (e.g., fisheries staff), initial on-site data collection may begin immediately.

Site Examination and Data Collection

The aquatic environment and surrounding terrain may vary dramatically, and human safety is paramount. Although you may be well prepared in terms of equipment, it is folly to investigate water bodies alone without firsthand knowledge. Local fisheries personnel generally have good information about the regional terrain and should accompany the investigation. Some means of contact with a central control point, with details of the investigator's whereabouts and expected contact times, should be prearranged.

Collection of on-site data should begin as soon as possible after arrival. Box 14-2 lists some basic equipment that may be useful for such investigations.

Attempt to circumscribe the affected area in order to estimate the extent of the problem and investigate it thoroughly. In freshwater environments this involves heading upstream beyond the boundaries of the problem area initially and then at least an equal distance downstream. In the marine environment, inspect nearby coastal areas and waters. Evaluate the main use of the surrounding area, which could be agriculture, aquaculture, forestry, industry, recreation, or urban. Take account of seasonal and routine practices related to such operations. Note any input, such as tributaries, drains, and sources of discharges, associated with the

Box 14-2**Basic Equipment for Disease Investigation in Aquatic Environments**

Battery-operated microscope, pH meter, and oxygen meter
 Boat availability
 Bottles for water and postmortem samples
 Camera
 Cell (mobile) phone and contact phone numbers
 Disposable gloves, paper towels, and wipes
 Electrofishing equipment
 Formalin
 Glass slides and coverslips
 Global positioning system (GPS)
 Ice container
 Life jacket
 Light source
 Maps
 Nets (various sizes)
 Notebook and pencil
 Plates and sterile loops for microbiology
 Postmortem dissection instruments
 Receptacles to hold fish
 Ruler and tape measure
 Thermometer
 Waterproof clothes, including long-legged boots
 Water-sampling equipment
 Weighing scales

surrounding land use. If necessary, obtain records regarding discharges and factory activities.

Check for unusual changes, such as land subsidence, and examine surrounding vegetation for evidence of death of other animals or abnormal behavior, such as snails having exited the water. Check for color changes in the water and nearby surrounding land, and note the presence of any pungent smells. Take photographs from various angles, and obtain relevant water and sediment samples from different locations, including control samples, to help avoid misinterpretation of natural variation in measured parameters. Note and photograph labels of chemical contents from any rubbish dumped near the site. Ascertain recent weather conditions, and inquire about recent changes in water levels and land use. Also, obtain past water-quality data for the area, if available.

Clinical Examination

In examining the affected population, note the general state of the fish. If they are dead, note any decay and changes in their general condition that may help in estimating the duration of the ongoing problem. Also

note the physical appearance: gills flared, mouths agape, twisted spines, excess mucus, and gross external lesions. In live fish, note their color and fin position, and assess any unusual behavior, such as loss of equilibrium or attempts to exit water.

Decide which fish are most representative of the overall problem and should be collected for further examination. In wild fish, one may assume that any causative disease agent will be present in affected fish at a high prevalence rate. For this reason, in the initial stages of a disease investigation, a thorough examination of a small representative sample is more informative than a select examination of large numbers of fish. A sample size of five is a good starting point. Samples are then taken based on species and age or size. If possible, samples from early-stage and late-stage disease are useful. Thus, in any one investigation, a number of samples of five fish may be taken.

Gross Postmortem Examination and Sample Collection

Some tasks should be done on-site during the gross postmortem examination (Figure 14-1). Other samples may be appropriately stored for further laboratory testing. Parasitologic examinations and swabs for microbiologic culture provide more meaningful results when taken from fresh fish, so sample sick fish, which are humanely euthanized, on-site. If sick fish cannot be caught, sample the freshest dead fish.

Weigh and measure each fish, and take skin scales for aging, if possible. Examine skin scrapes, gill squashes, and intestinal smears for parasites. Some parasites (e.g., *Argulus* spp.) tend to abandon fish as soon as they are removed from the water, and if care is not taken, the extent of such infestations may be underestimated.

Open the fish using a sterile technique, and look for any abnormal swellings, accumulations of fluid, hemorrhages, adhesions, or other lesions. Take swabs from the posterior kidneys and any visible lesions for culture. Make impression smears of individual organs and lesions for cytologic examination, where appropriate. A selection of tissues and visible lesions that include normal surrounding tissue should be placed in formalin for histologic processing and examination (Box 14-3). Collect further tissue samples, or freeze some whole fish, for toxicologic and viral testing (Box 14-4).

Open “small fry” from vent to gills, and store in formalin for histologic examination. Also, collect whole fry for microbiologic testing after maceration.

SELECTED DISEASES

Disease causes may be classified into the following groups: environmental, toxic, parasitic, bacterial, viral, and fungal. The following sections provide examples of diseases found in a wide geographic range.



Fig 14-1 Note external lesions during gross postmortem examination. (See Color Plate 14-1.)

Box 14-3**Fish Tissues to Sample for Histology**

Central nervous system
 Gills
 Heart
 Intestine, including pancreas if present
 Kidney (anterior and posterior)
 Liver
 Muscle
 Skin
 Spleen

Box 14-4**Fish Tissues to Sample for Virology and Toxicology**

Central nervous system
 Kidney
 Liver
 Muscle

Environmental Causes

The major environmental cause of fish kills is *oxygen deficiency*. Oxygen makes up approximately 21% of the atmospheric volume, but it is only sparingly soluble in water, and its availability is the most common limiting factor to fish life. The dissolved oxygen concentration in natural waters is a consequence of diffusion to and from the atmosphere, plus the oxygen produced from photosynthesis by aquatic plants and phytoplankton, less the oxygen consumed by respiration of living organisms in the water.

Variations in the amount of oxygen diffusion may result from changes in movement of air by winds or water turbulence. Oxygen concentrations are usually near saturation in the surface water of unpolluted rivers, lakes, estuaries, and oceans. In deep waters, oxygen levels are naturally low because biologic activity consumes oxygen, and aeration at depth is negligible. Oxygen produced by photosynthesis stops at night, but oxygen is consumed by plants as they respire in the dark. Therefore, dissolved oxygen levels may vary significantly during any 24-hour period, being highest in late afternoon and lowest at sunrise.

Oxygen saturation concentrations range from 6 to 15 mg/L, depending on other factors, such as salinity, temperature, and pressure. Fish will die from anoxia when dissolved oxygen levels reach a critical concen-

tration. This critical concentration varies with species, size, activity, and other environmental conditions. The U.S. Environmental Protection Agency (EPA) recommends a dissolved oxygen concentration of 5 mg/L for maintenance of a healthy fish population.³

Three types of natural oxygen deficiency occur: summer oxygen deficiency, winter oxygen deficiency, and turnover.

Summer Oxygen Deficiency

Increased water temperatures during periods of hot weather decrease the available dissolved oxygen. Other seasonal factors, such as decreased oxygen diffusion at the water surface in calm weather, low overall water exchange and volume in periods with low rainfall or drought, and pollution, also add to the difficulties. Summer oxygen deficiency occurs in the early-morning hours before sunrise. Large fish with high oxygen requirements are found dead, and small fish may be seen swimming lethargically and gasping for air in shallow water. Deaths may cease during the day when oxygen levels rise, but the problem continues the following night. Early-morning oxygen levels may be less than 1 mg/L, accompanied by a pH between 6 and 7. The concentration of ammonia and free carbon dioxide may also be raised. The water smells of sour cabbage and varies in color from pea green to dark brown because of dead algae and decaying vegetation, which may be seen on microscopic examination.

Winter Oxygen Deficiency

In winter oxygen deficiency, ice prevents oxygen diffusion from the atmosphere, and snow blocks light essential for photosynthesis. Mortalities may occur at any time of day, and other indicators are the same as for summer deficiency.

Turnover

The turnover phenomenon occurs in shallow or chronically polluted water bodies when anoxic water and decaying organic matter are churned by strong winds, hailstorms, or heavy rain. This increases biologic oxygen demand and decreases available oxygen.

Toxic Causes

The mechanisms of action of toxins are varied and complex, and their effects are determined by their

chemical composition, biodegradability, and rate and amount released into the environment, as well as other factors. The effects of toxins may be direct or indirect and acute or chronic. *Direct effects* include poisoning related to the mechanism of action of the compound, as seen in organophosphate poisoning, which affects the nervous system. *Indirect effects* of a toxin on a population of fish include oxygen deficiency, immune deficiency, and starvation from removal of the food source. *Acute toxicosis* usually occurs immediately on contact with a toxin, but in cases in which toxins bioaccumulate in fish tissues and are later released, the acute effects may be delayed. *Chronic toxicosis* is likely to compound and be compounded by many other environmental and pathogenic causes of health problems and may be difficult to diagnose accurately. High incidences of tumors have been seen in fish inhabiting heavily polluted marine waters, although it has proved difficult to obtain scientific evidence to establish the cause.

When toxicosis is suspected, diagnosis depends on selective collection and proper storage of water, sediment, and tissue samples and specific analysis. Reference material should be consulted.

Oxygen-Depleting Pollutants

Oxygen-depleting pollutants generally come from domestic sewage or factory and farm effluents. They may cause immediate oxygen deficiency by increasing the biologic oxygen demand or decreasing oxygen production by destroying phytoplankton and plant life. In delayed cases, leaching of top-dressed fertilizers from nearby land provides nutrients for plant and bacterial growth. This supports a larger population of fish life for a time. However, the system is prone to collapse when the increased biologic oxygen demand exceeds oxygen availability. Monitoring water chemistry may indicate an impending problem. Decreases in the dissolved oxygen level overnight to critical concentrations and wide variations of pH during the day are warning signs, as is an increased prevalence of other diseases. A flush of algae may compound the picture directly by producing toxins.

Metals

Copper, iron, manganese, mercury, lead, and zinc discharged from mining operations and industry are the more common metal toxins affecting fish. High levels of metals may also be found in water permeating underground seams of metal-bearing rock. Solubility is affected by water hardness, alkalinity, pH, and tem-

perature, and toxic levels of metals thus depend on water chemistry. Metal toxicity causes skeletal deformities, loss of reproductive activity, increased susceptibility to infections, and death. Histopathologic changes may be seen in liver, kidney, gill, the central nervous system (CNS), and other tissues where metals bioaccumulate. People who eat such fish are at risk of metal poisoning, and safety margins have been established for consumption of fish derived from areas where metal concentrations in the water are known to be high.

Toxic Gases

Ammonia, hydrogen sulfide, chlorine, and chlorine derivatives are the most common gases that are toxic to fish. They are released from animal waste, industrial and domestic pollutants, water treatment plants, and decaying matter. Supersaturation of water with oxygen and nitrogen may also be toxic.

Ammonia

The concentration of un-ionized ammonia, the most toxic form of this compound, increases as pH rises. Acute ammonia toxicity causes hypertrophy of gill epithelia and separation from capillary vessels, with an immediate effect on respiration. Chronic toxicity causes hyperplasia of gill epithelia with clubbing of lamellae, which eventually leads to occlusion of the gills, resulting in life-threatening respiratory crises.

Ammonia is naturally metabolized to nitrites and then to nitrates. High levels of ammonia in water may overload this pathway and lead indirectly to nitrite toxicity, which causes methemoglobinemia in fish.

Hydrogen Sulfide

Hydrogen sulfide toxicity is associated with sewage pollution or chemical contamination. The gas may also be released at toxic levels when anaerobically decomposing sediments are naturally or mechanically disturbed. The characteristic "rotten egg" smell is easily recognized, but the gas, which is highly soluble, quickly dissipates. It causes hyperplasia and clubbing of gill lamellae and diffuse vacuolar degeneration and necrosis in the liver.

Chlorine and Chlorine Derivatives

Chlorine-associated toxicities are generally related to the release of treated water from sewage plants and industries. The EPA recommends that effluents have

no more than 0.03 mg/L of residual chlorine.² Fish will try to avoid a contaminated area, but when affected, they lose equilibrium and die after exertion.

Supersaturation of Oxygen or Nitrogen

Supersaturation of oxygen or nitrogen may cause “gas bubble disease” in fish. In wild fish this occurs downstream when pressurized water is released from dams. Small bubbles of gas block the blood flow in capillaries, and larger gas bubbles are visible in the skin and eyes.

Chemical Pollution

The information section of chemical products available for domestic or commercial use usually contains the warning “Harmful to fish.” Indiscriminate disposal or dumping of chemicals can affect fish populations.

Oils, phenolics, and polychlorinated biphenyls (PCBs) produced by industry may end up in the aquatic environment. Oil spills cause extensive damage to aquatic life when oil covers fish, resulting in suffocation, or is absorbed and poisons the fish.

Agricultural products, such as pesticides for animals and plants and herbicides, generally adsorb onto sediment in the water or bioaccumulate in tissues, especially fat, after ingestion. Therefore, recent pollution events may be diagnosed by examination of water and sediment samples, whereas long-term exposure may be discovered by analysis of tissues.

Pesticides include chlorinated hydrocarbons such as dichlorodiphenyltrichloroethane (DDT), organophosphates, and carbamates. Some of these compounds bioaccumulate in fat tissues. No effects may be seen until the fish stop feeding in the winter and use up their fat stores, at which stage toxins are released and

act directly on the fish. DDT toxicity may also indirectly deplete the available food resources, and dead copepods, zooplankton, and insect larvae may be seen during field investigation. Starved fish are predisposed to numerous other pathogens. Organophosphates and carbamates act directly on the nervous system by inhibiting acetylcholinesterases. Organophosphates and carbamates may also indirectly affect available food supply. Affected fish may appear darker, are lethargic, and may develop tetany and spinal deformities.

Herbicides, which may remain in sediments for months, cause oxygen deficiency by destroying plants and phytoplankton in the aquatic environment.

Parasitic Causes

A myriad of parasites affect fish, ranging from amebae to crustaceans.¹ Parasites have been found to be more prevalent in polluted waters.⁴ Infestations may also rise in warmer weather, which may result in significant mortalities, especially in young fish. Parasites predispose fish to secondary infections, and conversely, many sick fish are heavily parasitized.

Argulus spp. are obligate macroscopic branchiuran copepods that parasitize the skin and buccal cavity of fish. Marine argulids have not often been associated with disease, but in slow-moving freshwater lakes, outbreaks have been associated with deaths of multiple species, age groups, and sizes of fish. Argulids may be identified by the naked eye (Figure 14-2). They repeatedly pierce the skin with a sting, injecting a toxic substance. As few as three argulids may kill small fish.¹ In larger fish, wounds become necrotic and ulcerated and secondarily infected with bacteria and fungi.

Costia spp. are small pear-shaped protozoans that parasitize the skin and gills of freshwater fish. *Costia* spp. have been found in salt water in farmed fish. Although similar in size to skin cells, they are easily distinguished on fresh smears by their jerky, spiraling motility. Irritation leads to epithelial hyperplasia of the skin and gills. Severe gill lesions lead to poor gas exchange, and death follows oxygen deficiency during growth of fish or an increase in population size. In polluted waters where gills are already compromised, a bloom of *costia* may be sufficient to kill the fish.



Fig 14-2 Argulids can be identified by the naked eye. (See Color Plate 14-2.)

Bacterial Causes

Many bacteria cause disease in fish. One major disease-causing group of bacteria is the aeromonads.

Aeromonads are gram-negative, rod-shaped bacteria found in aquatic habitats and associated with skin ulceration or death following septicemia. The two most important in terms of disease are *Aeromonas salmonicida* and *A. hydrophila*.

Aeromonas salmonicida

All species of freshwater and marine fish are considered susceptible to *A. salmonicida* organisms. Although most common in salmonids, disease has been reported throughout the world in cyprinids (carp), ictalurids (catfish), and labridae (wrasse). Other species affected include bream (*Abramis abramis*); dace (*Leuciscus leuciscus*); American, European, and Japanese eels (*Anguilla* spp.); roach (*Rutilus rutilus*); small-mouthed bass (*Micropterus dolomieu*); and tench (*Tinca tinca*). Within the salmonids, there are species differences in their predisposition to the disease, with salmon more susceptible than trout, and brown trout (*Salmo trutta*) more susceptible than rainbows (*Oncorhynchus mykiss*).

Aeromonas salmonicida does not grow or multiply well in water. Molecular diagnostics have shown that many populations of salmonids latently carry the organism, and disease outbreaks have been associated with various stress factors. For example, during the spring run of anadromous salmonids, which follows an arduous ocean migration and evasion of fishermen, a natural fall in freshwater levels halts the progress of the salmon upstream. Fish accumulate in deep, slow-flowing ponds. Under these conditions, the further stress of rising water temperatures, overcrowding, and poor oxygen levels may lead to disease outbreaks. Usually, large fish die with signs of septicemia, and anglers deprived of their quarry readily report the problem.

The clinical signs of infection vary. Fingerlings, parr, and smolts may die without any outward signs. Some fish may show slight exophthalmia, lethargy, congestion around pectoral and pelvic fins, hemorrhagic gills, and bloody discharge from the vent or nares. If the course of the disease is more prolonged, the organism may cause large areas of muscular necrosis, resulting in soft, palpable areas or boil-like swellings; thus the name *furunculosis* is common. Furuncles may burst and release reddish fluid and large numbers of bacteria that spread the infection. Lateral transmission during an outbreak is through infected water and infected fish contact. Fish that survive may show some evidence of scarring from ruptured furuncles.

Various atypical strains of *A. salmonicida* have been associated with skin ulcers, mostly in nonsalmonid fish. These vary from white discoloration or shallow

hemorrhagic ulcers to deep lesions that expose muscle or bone, which may be secondarily infected with fungi. Other bacterial systemic infections often occur and compound the pathology.

Typically, *A. salmonicida* may be cultured on tryptone soya agar, where it produces a brown pigment. Atypical strains may be nonpigmentary. They are fastidious organisms, and enriched media such as 5% bovine blood agar may be needed for primary isolation.

On postmortem examination of fish with furunculosis, evidence of environmental stressors such as net damage may be seen. Large areas of soft, fluid swellings may be palpated or visualized in muscles. Some of the carcasses may appear septicemic, with hemorrhages in the viscera and muscles, necrotic kidneys, pale liver, and enlarged spleen. *A. salmonicida* may be readily cultured from skin lesions or kidneys. On histologic examination of kidneys from affected fish, colonies of bacteria may be seen.

Aeromonas hydrophila

Aeromonas hydrophila is another gram-negative rod that may affect all species of freshwater fish. It is a common secondary invader. The organisms survive and multiply in waters where there are high levels of organic matter and sewage. Some strains tolerate brackish water. Thus the significance of culturing *A. hydrophila* depends on the clinical signs, the site of isolation, and the number of isolations from a sample of affected fish.

Disease may be precipitated by low oxygen concentrations, nitrite-induced methemoglobinemia, rapid temperature changes, other bacterial or parasitic infections, stress associated with spawning, decreased immunity, and physical trauma. For example, infection following physical trauma has been seen in bream fishing, where keep-nets weighted down with stones are used to hold the fish caught. The fish are released after the weight tally is recorded at the end of the day, to be fished for on another day. In these situations, diffuse, large hemorrhagic skin ulcers are believed to be related to repeated trauma in the nets causing skin damage and predisposing to *A. hydrophila* infection.

Disease may occur in individuals or in epidemics with variable mortality rates. Fish may be found with septicemia, as described for *A. salmonicida* infection. Abdominal distention is sometimes seen. Skin ulcers may be found anywhere on the body and range from shallow, hemorrhagic or grayish lesions to extensive necrosis of skin and muscle with overlying fungal infection. The organism may also cause tail or fin rot and gill and eye lesions.

Diagnosis is by culture and identification. *A. hydrophila* may be cultured on nutrient agar from blood, internal organs, and ulcers. Colonies are cream colored, round, and shiny.

On postmortem examination there is kidney necrosis. Anemia and hepatic damage may be found in chronic cases. Histology shows extensive congestion, hemorrhage, and tissue necrosis.

Viral Causes

Numerous types of viruses have been isolated from fish. In general, most viral infections are species specific. Although much is known about some fish viruses, especially those that affect farmed fish, much of the scientific evidence for the role of viral infections in wild fish disease is based on single case reports.

Lymphocystis is a well-studied, chronic, nonfatal viral skin disease recognized worldwide and occurring in many warm-water and cold-water fish in both fresh and marine environments. It is caused by an iridovirus. Infection occurs in fish of any age, although it is more common in young fish. Fish develop gross, creamy to gray, tumorlike nodules on the body surface over a protracted period. Lesions are unsightly, and the appearance of grotesquely disfigured fish results in the problem being reported (Figure 14-3). Lesions heal spontaneously, leaving little evidence of previous infection.

The lymphocystis virus causes a unique cellular hypertrophy with massive enlargement of both cytoplasm and nucleus of infected dermal fibroblasts. Single cells may expand up to 2 mm in diameter.

Transmission occurs in nature by exposure of wounds to waterborne virus or ingestion.

Although infection may be presumptively diagnosed by the gross appearance, histology or virus

isolation is required to differentiate the lesions from some parasitic nodules or tumors. On histology the lesions consist of individual hypertrophied cells. A hyaline layer thickens the cell membrane, and some mixed inflammatory cells are seen around the periphery. Horseshoe-shaped, basophilic, cytoplasmic inclusions are often apparent.

The lymphocystis virus is very stable and has been isolated in a variety of marine and freshwater fish fibroblast cell lines.

Fungal Causes

Fungal diseases are frequently found in fish. They cause gill, skin, and eye lesions in all ages of fish and may also infect eggs. The most common fungal disease in freshwater fish is *saprolegniosis*. The main species associated with disease are *Saprolegnia ferax* and *S. diclina-parasitica* complex. They are ubiquitous saprophytes of soil and water that can affect an extensive host range, including carp, catfish, eels, and salmonids.

In most cases, fungal infections are indicative of other primary problems, but once established, they may be the cause of death. Predisposing factors include a drop in water temperature, trauma, parasitic or bacterial infection, nutritional deficiencies, chemical or other irritants, and increased corticosteroid levels related to stress or hormonal changes in the fish. Infection is spread by release of spores into the water. Saprolegnial spores are inhibited by even moderate salt concentrations.

Fungal infections are generally recognized as cotton-wool-like growths on the skin and gills in live fish. On removal from the fish, the fungi collapse into a slimy mat of varying colors, depending on the substances trapped within. Acute infections begin as small foci of epithelial erosion, and fungus may spread over the body within 24 hours. Chronic infection may cause large, deep ulcers that expose the muscle. Affected fish are lethargic and die. The severity of the disease is related to the surface area of the fish affected, and death generally results from impaired osmoregulation, although smothering of the gills may lead to rapid death.

Dead fish are fertile substrates for fungal colonization, so diagnosis should be limited to live fish. Fungal identification is based on the morphology of various life cycle stages and generally requires expert knowledge. However, observing broad, aseptate hyphae in freshwater fish may allow a presumptive diagnosis.

Infection may be confirmed on histologic examination; usually a mild lymphocyte and macrophage



Fig 14-3 Lymphocystis causes unsightly, tumorlike nodules. (See Color Plate 14-3.)

infiltrate is associated with the fungal hyphae. Inflammatory cells are increased in the presence of other pathogens such as bacteria. Occasionally, fungal hyphae may be found penetrating internal organs.

CONCLUSION

There is much to learn about the epidemiology of disease in wild fish populations. Although a major difficulty is the inability to provide treatment, improved understanding of the multifactorial pathogenesis of disease occurrence and the reporting of scientific investigations could help determine new methods of disease prevention.

Acknowledgments

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Color Plate 14-1 Note external lesions during gross postmortem examination. (For text mention, see Chapter 14, p. 114.)



Color Plate 14-2 Argulids can be identified by the naked eye. (For text mention, see Chapter 14, p. 117.)



Color Plate 14-3 Lymphocystis causes unsightly, tumorlike nodules. (For text mention, see Chapter 14, p. 119.)

CHAPTER 15

Spring Viremia of Carp Virus

GREGORY A. LEWBART AND RAGHUNATH SHIVAPPA

Spring viremia of carp (SVC) is a reportable disease caused by a rhabdovirus (*Rhabdovirus carpio*) frequently referred to as spring viremia of carp virus (SVCV). Until recently (2002), SVCV had not been found in North America; it is considered a foreign animal disease (FAD) in the United States. In summer 2002 the disease was identified in koi (*Cyprinus carpio*) from a North Carolina fish farm that also owned pond facilities in Virginia.¹⁰ Subsequently, cases have been confirmed in other states. Warg et al review the U.S. cases from 2002 to 2004, summarize the diagnosis and pathophysiology of SVCV, and describe genetic relationships among various viral strains.²³

Mortality from SVCV in carp may reach 100% but is frequently much less. Younger fish are more susceptible than older fish, and infected fish typically present with abdominal ascites and multiple-organ hemorrhages. Transmission is horizontal, and most cases occur in the spring or early summer, when the water begins to warm but remains below 15°C.

Diagnosis is usually made with viral isolation (from spleen or caudal kidney) and serum antibody titers. Diagnosis should be confirmed with virus neutralization.¹⁵ The disease is not restricted to koi and actually may affect several carp species and some other cyprinids (Box 15-1).^{3,7} All suspect cases should be necropsied and the U.S. Department of Agriculture (USDA) contacted for proper routing of diagnostic samples. Confirmed cases must be reported to the USDA.

A complete summary of the disease and diagnostic procedures may be found on the Office International des Epizooties (OIE) website.¹⁶ It is important to note that SVCV-infected fish may also have a gram-negative bacterial infection.¹⁸

CLINICAL SIGNS

Early in the course of SVCV, affected fish may appear weak and may congregate in areas of slow-flowing water. Infected fish may present with a variety of clinical signs, including but not limited to lethargy,

ascites, exophthalmia, pale gills, overall darkening of the body surface, persistent fecal casts, skin and branchial hemorrhages, and distention/protrusion of the vent² (Figure 15-1).

On necropsy, affected fish may have generalized edema (the fluid may be sanguineous), swim bladder (and other organ) hemorrhages, and intestinal inflammation. The gastrointestinal tract may contain mucus and not ingesta.¹⁴

DIAGNOSIS

A variety of serology and polymerase chain reaction (PCR)-based assays are available to veterinarians and fish health professionals for documenting the presence of SVCV. The World Organization for Animal Health (OIE) set the international standards for diagnosing SVCV in 2000. The diagnosis of SVC in clinically infected fish may be carried out by rapid immunologic assays such as direct immunofluorescence (IF) or enzyme-linked immunosorbent assays (ELISAs) on infected tissues. However, OIE recommends that the results from these tests be further confirmed by virus isolation in cell culture (Figure 15-2), followed by virus neutralization (VN) test. In situations in which it is not possible to isolate the virus (e.g., decomposed clinical samples), clinical signs of SVC and a positive direct IF test or ELISA are considered sufficient to initiate control measures.

Screening of clinically healthy fish (asymptomatic) is strictly based on virus isolation in cell culture followed by VN for confirmation. While awaiting the VN results, however, positive results from rapid methods (e.g., IF, ELISA) are sufficient to initiate fish health control measures.¹⁶

Molecular techniques, including reverse-transcriptase PCR (RT-PCR), have been developed for the identification of SVCV. However, RT-PCR may be quite dependent on the conditions under which it is run and may be highly subject to laboratory contamination by previous PCR products, yielding false-positive results. Although OIE recommends RT-PCR as a confirmatory

Box 15-1

Fish Species Susceptible to Spring Viremia of Carp Virus (SVCV)

Naturally Susceptible Species

Common carp (*Cyprinus carpio*)⁸
 Grass carp (*Ctenopharyngodon idella*)¹⁹
 Silver carp (*Hypophthalmichthys molitrix*)²¹
 Bighead carp (*Hypophthalmichthys nobilis*)²¹
 Crucian carp (*Carassius carassius*)¹³
 Koi (*Cyprinus carpio*)⁸
 Goldfish (*Carassius auratus*)¹²
 Tench (*Tinca tinca*)⁵
 Orfe (*Leuciscus idus*)⁵
 Minnow (*Phoxinus phoxinus*)³
 Sheatfish (*Wallago attu*)⁹

Experimentally Susceptible Species

Golden shiner (*Notemigonus crysoleucas*)¹⁰
 Roach (*Rutilus rutilus*)¹¹
 Chub (*Leuciscus cephalus*)³
 Barbel (*Barbus barbus*)³
 Dace (*Leuciscus leuciscus*)³
 Bream (*Abramis brama*)³
 Zebra danios (*Danio rerio*)²⁰
 Pike (*Esox lucius*)⁴
 Guppy (*Poecilia spp.*)¹
 Pumpkinseed (*Lepomis gibbosus*)⁶

test for the diagnosis of SVCV, well-established techniques (e.g., virus isolation) are specified as standard screening methods. It is highly recommended to include adequate positive and negative controls and to use extreme caution when RT-PCR is used as a method of choice for diagnosis of SVCV. Box 15-2 provides contact information for SVCV testing laboratories approved by the USDA Animal and Plant Health Inspection Service (APHIS).

SAMPLE COLLECTION

For clinically affected fish, a minimum of 10 moribund fish or 10 fish exhibiting clinical signs of SVCV must be collected. Fish should be alive when collected and should be sent to the laboratory alive, or killed and packed separately in sealed aseptic refrigerated containers or on ice. Depending on the size of fish, whole fish (body length, 0-4 cm) or the entire viscera, including kidney and encephalon (body length, 4-6 cm), should be collected.

If the fish is larger, liver, spleen, and encephalon should be collected aseptically. Samples from 10 diseased fish should be combined to form pools of a

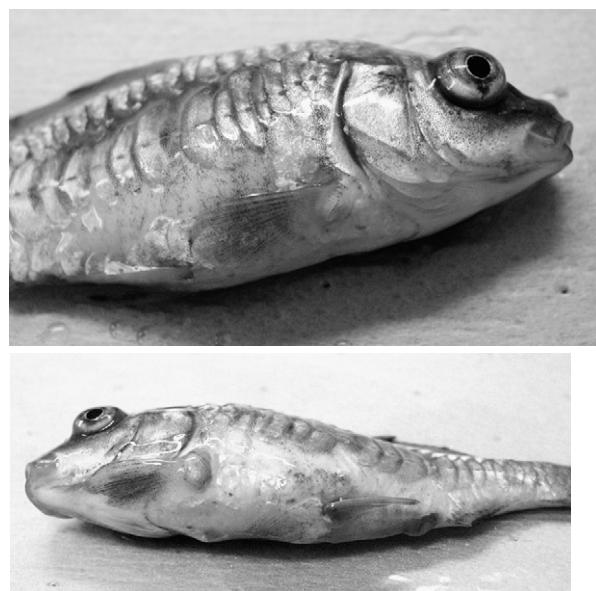


Fig 15-1 Common carp (*Cyprinus carpio*) showing external clinical signs of spring viremia of carp (SVC) after experimental infection with SVC virus. Note the exophthalmia, petechial hemorrhage of the skin, and inflammation of the vent. (See Color Plate 15-1.) (Courtesy Dr. Andy Goodwin.)

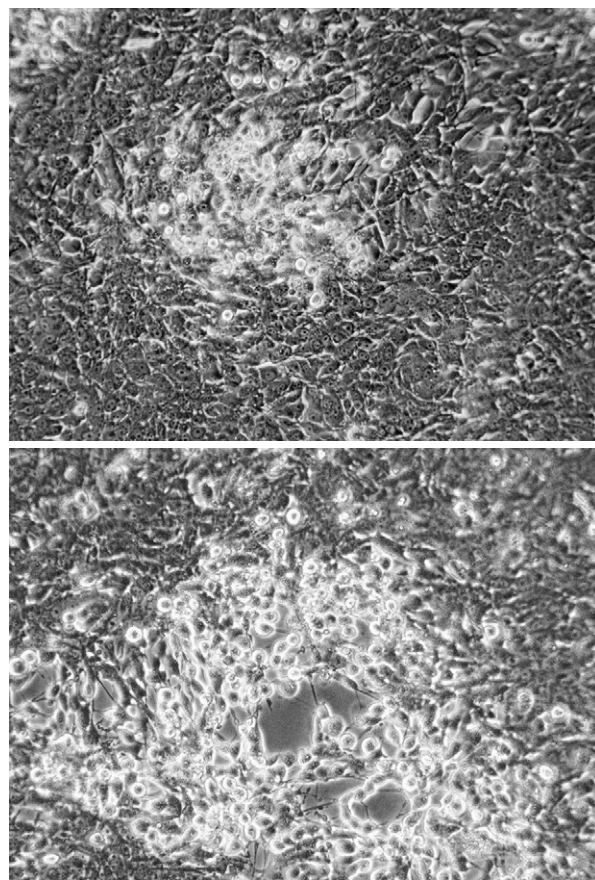


Fig 15-2 Cytopathic effect (CPE) induced by spring viremia of carp virus (SVCV) in epithelioma papillosum cyprini cell (EPC) monolayers. The CPE is characterized by widespread cell rounding followed by release of cells from the plate surface. (See Color Plate 15-2.) (Courtesy Dr. Andy Goodwin.)

Box 15-2

USDA-APHIS–Approved SVCV Laboratories**Maryland Fish Health Diagnostic Laboratory**

Department of Agriculture
8074 Greenmead Dr.
College Park, MD 20740
Phone: 301-935-6074

Micro Technologies, Inc.

41 Main St.
Richmond, ME 04357
Phone: 207-737-2637

Pennsylvania Animal Diagnostic Laboratory System

State Veterinary Laboratory
2305 North Cameron St.
Harrisburg, PA 17110
Phone: 717-787-8808

University of Arkansas–Pine Bluff

Cooperative Extension Program
1200 University Dr.
Pine Bluff, AR 71601
Phone: 870-543-8537

Washington Animal Disease Diagnostic Laboratory

College of Veterinary Medicine
PO Box 647034
Pullman, WA 99164-7034
Phone: 509-335-9696

maximum of five fish per each pool (each pool should not weigh more than 1.5 g). Pools of organs should be placed in sterile vials and stored at 4°C until virus extraction is performed at the laboratory. Virus extraction should optimally be carried out within 24 hours after fish sampling, but is still acceptable for up to 48 hours.

Organ samples may also be transported to the laboratory by placing them in vials containing cell culture medium or Hanks' balanced salt solution (HBSS), with added antibiotics to suppress the growth of bacterial contaminants (one volume of organ in at least five volumes of transportation fluid). Antifungal compounds may also be incorporated into the transport medium. Serum or albumin may be added to stabilize the virus if the transport time will exceed 12 hours.¹⁶

For detecting asymptomatic carriers, tissue samples of kidney, spleen, gill, and encephalon should be collected. Samples may be combined as pools of no more than five fish/pool, for a total weight of about 1.5 g. Depending on the population size, fish collection must encompass a statistically significant number of

Table 15-1

Samples Required for Detection of SVCV-Infected Fish*

Lot or Population Size	Sample Size
50	35
100	45
250	50
500	55
1000	55
≥2000	60

Data from Ossiander J, Wedemeyer G: *J Fish Res Board Can* 30:1383-1384, 1973.

*95% confidence interval.

specimens. The sampling should be designed to enable detection, at a 95% confidence level, of infected animals (Table 15-1).

DISINFECTION

The disinfection protocol depends on the size, type, and nature of the materials and sites to be disinfected. When there is an active outbreak of SVCV, the infected stocks should be depopulated, and all areas that held the infected fish must be disinfected. The virus may be inactivated by formalin, ozone, sodium hypochlorite (bleach), organic iodophors, gamma and ultraviolet (UV) radiation, pH extremes of less than 4.0 or greater than 10.00, and heating at 60°C for 15 minutes.^{7,22} All equipment and tanks, raceways, and ponds should be disinfected. The USDA-APHIS also recommends that the incoming water to the farms be treated with sand filtration and UV radiation (Figure 15-3).

In the North Carolina/Virginia outbreak of SVCV, infected fish were reported from four earthen ponds that had experienced an approximately 10% mortality (15,000/150,000 koi). The farmer depopulated and drained these ponds before the confirmation of SVCV. Subsequently, all 202 ponds were drained, the fish were depopulated, and the pond base was treated with lime (calcium oxide/calcium hydroxide), as recommended by OIE (Figure 15-4).

Once an infection is reported from a facility, it must follow the recommendations described in the International Aquatic Animal Health Code and the *Diagnostic Manual for Aquatic Animal Diseases* by OIE to be declared free of SVCV.¹⁶ The USDA-APHIS implemented similar recommendations for the affected farm, as follows:



Fig 15-3 Sand filtration (A) and ultraviolet (UV) radiation (B) treatment units for the incoming water. This equipment was installed at the North Carolina/Virginia farms to prevent SVCV. (Courtesy Raghunath Shivappa.)



Fig 15-4 Disinfection of ponds with lime where SVCV-infected fish were collected. (Courtesy Dr. Rosemary Sifford.)

1. The farm should test negative for SVCV under an official health surveillance scheme for at least 2 years.
2. Water supply should be only from a spring, well, or borehole and must be free from wild fish.
3. The incoming water should not be connected to a watercourse, or a natural barrier should prevent the upstream migration of fish from downstream stretches of the waterway.

In addition, a surveillance program was also implemented in the area for 2 years for detection of SVCV. The study did not isolate SVCV from any of the sampling sites around the North Carolina/Virginia outbreak area.³

CONCLUSION

Spring viremia of carp is a serious and reportable disease of cyprinid fishes that was first confirmed in

North America in 2002. Veterinarians and other health professionals working with fish should be familiar with the clinical signs and signalment particular to this disease. Suspicious cases should be thoroughly worked up and, when necessary, reported to the appropriate authorities.

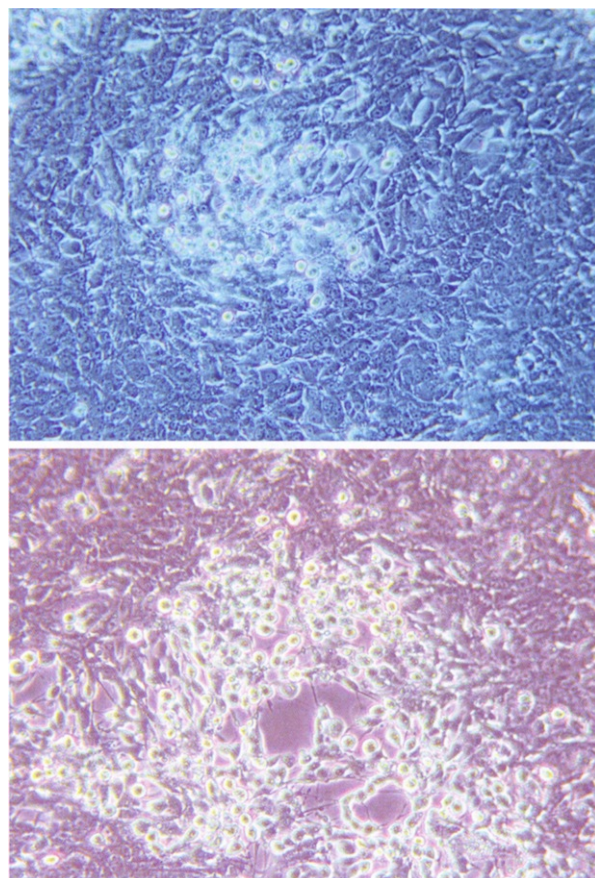
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Color Plate 15-1 Common carp (*Cyprinus carpio*) showing external clinical signs of spring viremia of carp (SVC) after experimental infection with SVC virus. Note the exophthalmia, petechial hemorrhage of the skin, and inflammation of the vent. (For text mention, see Chapter 15, p. 122.) (Courtesy Dr. Andy Goodwin.)



Color Plate 15-2 Cytopathic effect (CPE) induced by spring viremia of carp virus (SVCV) in epithelioma papillosum cyprini cell (EPC) monolayers. The CPE is characterized by widespread cell rounding followed by release of cells from the plate surface. (For text mention, see Chapter 15, p. 122.) (Courtesy Dr. Andy Goodwin.)

CHAPTER 16

Veterinary Participation in Puerto Rican Crested Toad Program

GRAHAM CRAWSHAW

The crested toad, or sapo concho (*Bufo [Peltophryne] lemur*), is one of eight endemic West Indian bufonids and is now found only on Puerto Rico. It has prominent supraorbital crests and an upturned snout. The toads are sexually dimorphic; females are 80 to 120 mm long and ash gray–white to charcoal black; whereas the males are slightly smaller, 70 to 80 mm long, with a brighter, yellow-green coloration (Figure 16-1).

First described in 1868, the Puerto Rican crested toad (PRCT) was distributed over much of the coastal karst areas of the island 100 years ago, but climatic change and loss of habitat resulting from land alteration for agriculture and commercial development seriously reduced the numbers of toads and threatened to eliminate the species altogether. The introduction of the marine toad (*Bufo marinus*) and the small Indian mongoose (*Herpestes auropunctatus*) to the West Indies has also had an impact on the endemic toad. Mongoose predation was a significant factor identified by radiotracking captive-reared toads released in Puerto Rico.

CONSERVATION

The PRCT had not been seen for more than 35 years until its rediscovery in 1965.⁵ No record of captive animals exists before 1980. Puerto Rico is a mountainous island, and only two isolated toad populations were known to exist: a northern population, now believed to be extirpated, and a southern population believed to be as low as 1500 to 2000 in the 1980s.⁶ These populations have been isolated since the Pleistocene, and they are managed in captivity as two genetically distinct groups. Because of its fossorial nature, the species is difficult to inventory. During the day, toads live

underground, usually entering secure, moist crevices or holes in the limestone karst. The largest known breeding population is found in Guanica Forest Reserve on the southwestern coast.

Typically, when the rainfall exceeds 18 cm over a 24-hour period, the adults emerge to breed at temporary ponds near the coastal beach. The breeding area is located adjacent to the sea, and inundation by seawater during hurricanes is a real threat. Before 1984 the breeding pond was drained to provide easier beach access. When this practice was stopped, it was discovered that toads were using this pond as a breeding site (Figure 16-2). The northern toad populations, found near Quebradillas, had bred in concrete walk-in cattle troughs together with *B. marinus*. Here, no more than 25 PRCTs had been seen at any one breeding episode. There has been no sighting of northern toads since 1992, and wild northern toads at this time are functionally extirpated. The remaining northern toads that now exist in zoos originate from a single breeding of the last pair of this race. In May 2006, tadpole descendants from these were released in newly constructed ponds near Arecibo, the first time in 15 years.

For several years, censuses in the Guanica Forest showed a population at the breeding pond of fewer than 300 toads, but in 2004 more than 600 adult toads appeared at the pond in three separate breeding events.¹ In 2005, four breeding events took place involving 2200 toads.

The PRCT species was considered suitable for a species survival plan (SSP) under the auspices of the Association of Zoos and Aquariums (AZA) in 1984 and was the first amphibian included in the SSP program. The 21 facilities currently holding 340 *Bufo lemur* manage approximately 400 captive animals as one population. Since the late 1980s, a captive breeding



Fig 16-1 Adult male Puerto Rican crested toad (*Bufo lemur*). (Courtesy B. Johnson.)

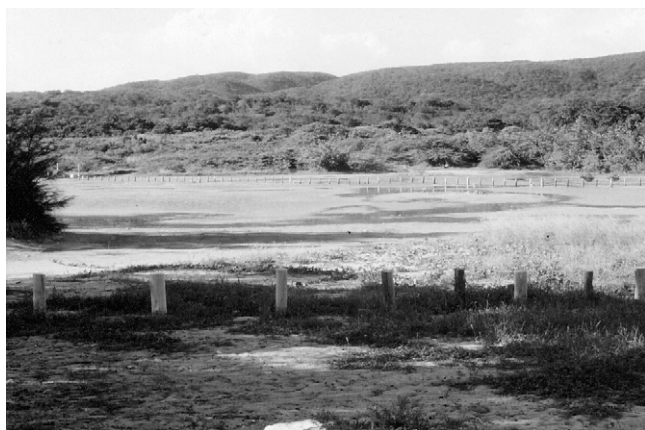


Fig 16-2 Breeding habitat in the Guanica Forest Reserve, Puerto Rico, in the dry season. (Courtesy B. Johnson.)

and release program has been undertaken to augment the wild population. Releases are only considered for suitable habitats beyond the range of the extant population. To date (2006), approximately 100,000 tadpoles hatched in North American zoos have been released in Puerto Rico.⁷ Additional breeding sites have been constructed, and in 2003, for the first time, adult toads grown up from captive-bred tadpoles were breeding at the new sites.

Veterinary involvement in the PRCT program has focused on reproduction, medical care, and pathology of captive animals and training of staff in the care of toads in Puerto Rico. Field projects are proposed, as described next.

Husbandry

As in other breeding programs for threatened species, captive populations of PRCT have been established

(1) to provide a reservoir of animals against catastrophic loss of toad in the wild, (2) to reinforce wild populations, and (3) to provide animals to establish new populations. The recovery program is a joint effort of zoos, the Puerto Rican Department of Natural Resources, and the United States Fish and Wildlife Service in Puerto Rico. The University of Puerto Rico provides staff for field projects. In zoos, PRCTs are displayed in terraria equipped with rock piles or rock and cemented caves to mimic their natural karst habitat. They are behaviorally reclusive, and hiding places are provided. Full-spectrum (Vita-lite, Duro-test, Philadelphia) and black-light (Sylvania, Danvers, Mass) bulbs are suspended directly over the tanks on a 12:12 light/dark photoperiod. Off-exhibit breeding animals are held in plain tanks with hollow polyvinylchloride (PVC) plumbing tubes to provide the tight, secure environment they prefer. Shallow water provides the ability to rehydrate. Feed requirements are the same as those of other equivalent anurans and typically include domestic crickets and other insects and newborn mice.

Reproduction

As noted, PRCTs are generally sexually dimorphic, and the gender of mature males may be confirmed by the presence of black nuptial pads on the "thumbs." Despite prebreeding conditioning with a period of cooling or drying, most pairs of PRCTs fail to reproduce spontaneously, and in almost all cases, exogenous hormones are used to promote reproductive behavior and success. Production also must be maximized and is timed to coincide with the rainy season in Puerto Rico when the tadpoles are released. Reproductive induction of anurans involves the use of human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone/luteinizing hormone-releasing hormone (GnRH or LHRH).

Several chemical analogs of LHRH exist, and not all work well in each species. The analog most often used for anurans is des-Gly (d-Ala) LHRH ethylamide (catalog #L4513, Sigma, St. Louis). To achieve natural reproduction, it is necessary not only to induce gamete production and release, but also to promote behavioral amplexus for fertilization of released ova. Experiments have shown that hCG injected into PRCT males is somewhat more effective than LHRH at inducing amplexus, although they were similar at promoting spermiation. Other studies have examined the need for preconditioning, and results have suggested that, in males at least, preconditioning is not a prerequisite

for successful gamete production. However, it apparently is not possible to induce ovulation in female PRCTs that have not been cooled or environmentally cycled, and hormone therapy will never be effective in the absence of mature ova in the ovary. In other anurans, ova maturation depends on an extended quiescent time and good nutritional status.

The timing of the injection is based on work that shows a peak in sperm production between 6 and 24 hours after injection. At the end of the cooling period for females, the toads are warmed back up to 28°C over 3 days. On day 2 the tanks are filled with 1 inch of dechlorinated, aged water. Audiotape toad calls are played throughout the day and into the night. As early as possible on the morning of day 3, males are injected with the LHRH analog ethylamide (Sigma), 0.1 µg/g subcutaneously (SC), or hCG (Chorulon, Intervet Canada, Whitby, Ontario), 4 IU/g.⁷

Female toads are also injected at this time with 0.1 µg/g LHRH. Eggs are usually laid overnight within 24 hours of amplexus or after hormonal injections. Females seem to be susceptible to fluid overload, and death from drowning may occur. The breeding period is a stressful time for toads, and deaths have occurred from infection, fluid overload, and glomerulopathy, perhaps because of intense mobilization of protein in this breeding period.

In some cases, pairs of toads will go into amplexus for variable periods but fail to deposit eggs. The use of hormones in toads that are not “ready” may result in internal laying in which ova are not collected by the oviducts and remain to decompose in the coelom, leading to toxic or septic coelomitis. I have also seen prolapse of the ovisac (oviduct) subsequent to egg laying, particularly if the eggs are not released completely.

Anesthesia

Puerto Rican toads have been anesthetized with tricaine (MS-222, Argent, Redmond, Wash). It was found that 2 g/L buffered to a pH with the same dosage of sodium bicarbonate produces an adequate level in 8 minutes or less. Toads are placed in a shallow solution with care taken to keep the nostrils above the water level. Once toads have reached the required level, they are removed for the anesthetic solution. Toads anesthetized with topical tricaine have been kept for an hour or more at cool temperatures, without the need for additional drugs. It is generally not necessary and may be harmful to keep them in anesthetic solution for

longer than induction. Local anesthesia is used for treatment of dermal lesions and microchipping (see following discussion).

Identification

Local anesthesia is used for inserting transponders (“PIT tags”). Toads are held, and the location for needle insertion is cleaned with sterile water, followed by the application of a small amount of lidocaine/prilocaine cream (EMLA, AstraZeneca, Wilmington, Del). The local anesthetic takes only a few minutes to be effective, and the chip is placed subcutaneously on the left side of the upper thorax beneath the overhanging parotoid gland. The opening in the skin is closed with tissue glue (e.g., Vetbond, 3M, St. Paul, Minn). The area is then rinsed with water to remove traces of the anesthetic, which is capable of causing general anesthesia in high doses in toads. In most cases the transponders remain at this location, although migration, even into the coelomic cavity, has been seen. Microchipping has been successful in toads as small as 30 g. Implanting with PIT tags means that all the toads may be housed together rather than in pairs, which reduces holding-space requirements. The ventral pattern may also be used to identify individuals.

DISEASES

Knowledge of the diseases of amphibians in general is increasing.¹⁰ There has been considerable research in the role of disease, and of chytridiomycosis in particular (see Chapter 17), in the decline of amphibian populations globally. There is no reason to believe that *B. lemur* is unique among amphibians from a disease point of view. However, the health of the wild population has never been studied systematically, a task made difficult by their highly secretive nature. PRCTs are typically only found during the breeding events. A health survey has been initiated, first encompassing the sympatric and more common *B. marinus*, to include screening for pathology, parasites, and pathogens such as chytrid fungus. The study will subsequently be extended to include *B. lemur*. In captive animals, most deaths have been sporadic, although several multiple die-offs have occurred in both adults and juveniles. These cases appear to have been caused by suboptimal husbandry rather than specific pathogens.

A review of 117 necropsies at the Toronto Zoo (TZ) from 1985 to 2002 revealed that the majority of toads

had two or more histologic diagnoses. Nineteen cases (16%) of systemic infectious disease were noted. Most of these (67%) were diagnosed with single- or multiple-organism septicemia. *Pseudomonas* was the most frequently isolated bacterial organism from septic animals. Eight cases (7%) of musculoskeletal disease were noted, six of which (75%) were a myopathy of unknown etiology. Histologic lesions included myodegeneration, increased granularity, and hypereosinophilia of the sarcoplasm, with variable inflammation ranging from none to lymphocytic.

Twenty-two cases (21%) of enteric disease were observed. Many of these were the overeating/gastric dilation syndrome, for which no other cause of death could be attributed. Other cases of enteric disease included hepatocellular degeneration, hepatitis, unspecified endoparasitism, and mixed-inflammatory cell enteritis, as well as ceroid accumulation in the intestinal wall.

Seven cases (6%) of integumentary disease were diagnosed. Three of these were mycotic dermatitis, from which organisms such as *Nigrosporum* and *Trichoderma* were isolated. The remainder included a bacterial dermatitis concurrent with septicemia, suggesting that the skin was the portal for the pathogens. Three cases of respiratory disease were diagnosed, including one case of aspiration pneumonia and two of heterophilic pneumonia. Two cases of trauma and one case of ammonia toxicity ("tank die-off") were noted.

Of the 117 cases, 29 (25%) were diagnosed with renal disease, principally glomerulonephropathy. Eight cases (7%) of interstitial nephritis and one case of cystitis were also diagnosed. The exact cause of glomerular disease is unclear. All except one of these cases were in adult animals. Many of the toads with glomerulopathies had other diseases that might have predisposed them to glomerular disease, the most common being myopathy, dermatitis, and sepsis. Immune stimulation associated with dermatitis or sepsis may ultimately cause renal immune complex disease.

Limb paralysis has been seen in several toads in the collection. Because many of these amphibians improve with treatment, necropsy has been sporadic. Lesions found in these animals include one case each of wallerian degeneration and peripheral axonopathy. In toads in which the forelimbs were principally affected, pathologic findings were minimal, and in retrospect these may have been cases of thiamine deficiency. Others with hind limb paralysis have shown spinal axonopathy. Possible causes of such a condition

include dietary (hypovitaminosis E or B), infectious (neuritis, myositis, myelitis), autoimmune, or genetic processes. One affected toad had an osteomyelitis of the spine. Several cases of spindly leg syndrome were seen.

Several toads were diagnosed with anasarca and ascites of unknown origin. Some animals had been recently placed in breeding tanks and presumably had some alteration in normal osmoregulatory function. In addition, one adult animal was diagnosed with a systemic myeloproliferative disease of possible granulocytic origin.

Infectious Disease

Viruses

No viruses have been identified to date in PRCTs. Die-offs in tadpoles have been investigated for the possibility of ranavirus infection but, to date, none has been detected by polymerase chain reaction (PCR).

Bacteria

As with all amphibian species, bacterial septicemia and dermatitis are of concern in *B. lemur*. Most of the bacterial species of concern are gram-negative opportunistic invaders. Organisms isolated from cases of sepsis and dermatitis include *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Bordetella*, *Citrobacter*, *Flavobacterium*, *Hafnia*, *Pseudomonas* (*Comamonas terrigena*), and *Serratia marcescens*. Multiple organ systems are often infected in toads with septicemia, including liver, kidney, spleen, and occasionally heart. *Acinetobacter* has been isolated from a toad with pneumonia. Four young toads died from necrotizing hepatitis, with mouth or tongue necrosis. A toxic or infectious etiology was suspected but could not be identified. Die-offs from *Aeromonas* septicemia have been seen in groups of both tadpoles and toads, usually secondary to poor water quality, high ammonia levels, or overcrowding. Ascites is the most common finding on gross pathology, but petechiae may also be seen. Suspected bacterial disease in PRCTs is usually treated with enrofloxacin (5-10 mg/kg SC q24h) and/or amikacin (5 mg/kg SC q24h).

Bacterial dermatitis, manifested by ulcers and necrosis, particularly on the head, has been seen after stressful events such as breeding. Lesions on females may be initiated by prolonged contact with the males during amplexus (Figure 16-3). *Pseudomonas* and other

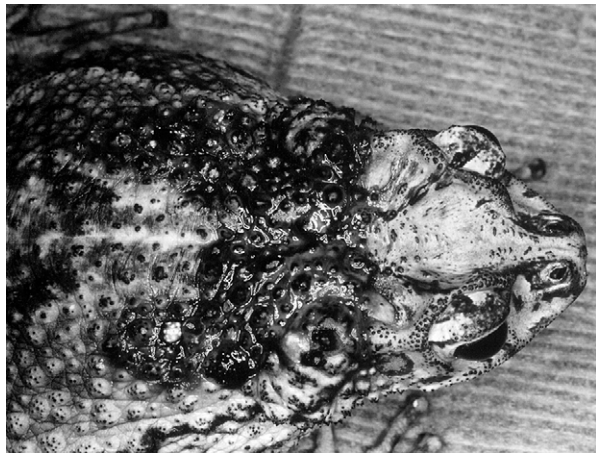


Fig 16-3 Bacterial dermatitis on dorsum of female Puerto Rican crested toad (PRCT) after amplexus.

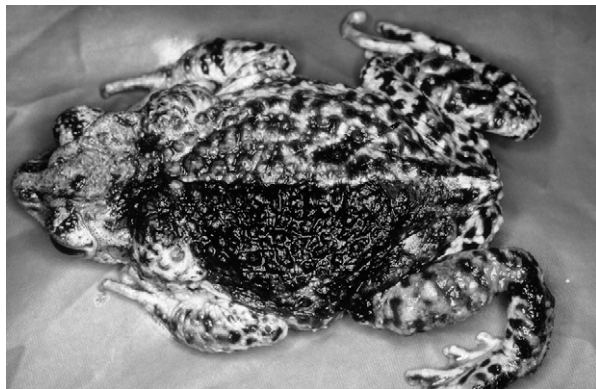


Fig 16-4 Nodules on lip of PRCT caused by infection with *Mycobacterium chelonae*.

gram-negative organisms are contributory. Treatment with systemic antibiotics and topical antiseptics is usually curative, although if untreated, the condition may progress to systemic disease, renal failure, and disturbances of water balance.

Mycobacterial infections have been seen very sporadically as wartlike nodules on the lips and are likely secondary to trauma (Figure 16-4). Another single animal developed mycobacterial infection in the stump of an amputated hind leg, from which *Mycobacterium chelonae* was cultured.

Fungi

Systemic fungal disease and fungal dermatitis are occasionally seen in the clinical setting. My group has seen superficial infection of the integument with *Nigrosporum*. Lesions are typically black, necrotic, and ulcerated, with occasional white, raised areas. At least two toads have developed a black, crusty



Fig 16-5 Fungal dermatitis on dorsum of PRCT.

covering on either the whole back or part of the back (Figure 16-5). *Trichoderma* was cultured, and hyphae were identified histologically interwoven in keratin accumulation in the skin. Treatment involved soaking the toads in a dilute emulsion of itraconazole or enilconazole for 5 minutes once daily for several days. Tadpoles occasionally also develop fungal infections, manifesting as multiple deaths within a tank, and an esophageal yeast infection and chromomycosis were seen in individual adults. No institutions have reported cases of chytridiomycosis (*Batrachochytrium dendrobatidis*), but to ensure no cases of infection, strict isolation from other potentially infected amphibians is essential. SSP protocols require dips and tadpole screening before release (see later discussion).

Parasites

Parasites do not appear to be a significant problem for the PRCT. One early examination of wild toads revealed trematodes (*Mesocoelium*), oxyurids, and other unidentified nematodes, as well as pentastomid larvae. Parasitic disease has been uncommon in captive *B. lemur*, but nematodes and other parasites may be acquired from other anurans in a zoo setting. A group of individuals at one zoo was diagnosed histopathologically with a *Balantidium*-like enteric parasitism. With no gross evidence of enteric disease or histologic evidence of inflammation, the pathogenesis remains unclear. Amoebic enteritis was diagnosed in one individual with an associated, predominantly mononuclear inflammation. As in other anurans, enteric protozoans are often found in PRCT fecal samples and postmortem colonic smears, especially in cases of diarrhea from any cause. Flagellates, nyctotherid ciliates, and the trophozoite and cystic forms of the multicellular flagellate opalinids are seen frequently.

Treatment of parasitic diseases has met with variable success, depending on the parasite in question. *Entamoeba* may be treated with metronidazole, 100 mg/kg orally (PO) every 14 days (q14d), or daily for 3 to 5 days, repeated in 14 days. Fenbendazole (100 mg/kg PO q14d), ivermectin (0.2-0.6 mg/kg SC/PO q14d), moxidectin (0.5 mg/kg SC), and praziquantel (10-20 mg/kg PO q14d) may be used alone or in combination for helminth infections. Treatment of intestinal protozoan infections by immersing toads in metronidazole solutions has been effective. Toads are placed in a bowl containing 200 mg/L of metronidazole injectable solution for 15 minutes once daily for 5 days. Coccidial oocysts have been found in some locations, but there has been no correlation with a disease condition.

Noninfectious Diseases

Calcium Deficiency

Pathologic fractures of the legs and spinal deformities (scoliosis) have been seen sporadically, indicative of inadequate calcium and possibly vitamin D intake. The provision of a balanced diet is a constant problem in feeding small amphibians, particularly juvenile animals. Powdering insects with supplements is a haphazard technique, and a reliance on one type of food may lead to long-term deficiencies.

Thiamine Deficiency

Thiamine deficiency was seen in several cohorts of 1- to 2-month-old toadlets, which developed a rigid paralysis of the forelimbs, held tucked back against the sides of the body; unresponsive eyes; head tilt; and apnea. Toadlets given calcium, glucose, and thiamine gradually returned to normal within 2 hours, but those given calcium and glucose alone showed no improvement. One given only thiamine was moving spontaneously within 30 minutes and had completely recovered by next morning. No cases have been seen after additional thiamine was added to the powdered supplement.

Overeating Syndrome

Young toadlets are voracious eaters and, given the opportunity, will fill their stomachs beyond capacity. Mortality has occurred when toadlets 3 months to 1 year old gorge themselves and are subsequently

exposed to water. The expanded stomach likely impairs vital functions such as respiration and circulation. Postmortem findings are limited to internal fluid accumulation and an expanded stomach. The condition has been avoided subsequently by carefully limiting the quantity of food offered to prevent overeating at this critical life stage.

Ceroid Accumulation

Ceroid accumulation in the intestinal wall in a cohort of young toadlets may have been associated with hypovitaminosis E.

Physical and Chemical Agents

Desiccation

In the wild, dehydration is a significant cause of mortality, particularly in tadpoles and in migrating juvenile toads. Rainfall is sporadic in much of their range, especially the southern areas; the ground is very porous; and the breeding ponds dry up at an alarming rate. In captivity, mortality has occurred in several groups as a result of manipulation of the environment for breeding purposes. Generalized desiccation may occur during prebreeding estivation or cooling, and careful monitoring of both toads and substrate is essential to prevent excessive dehydration.

Drowning

Conversely, when conditioned animals are rehydrated, excessive absorption of water into toads still experiencing renal shutdown may result in overhydration and death. It is recommended that rehydration be carried out gradually over at least 48 hours, and a diuretic (e.g., furosemide) may be given to toads that show a tendency to accumulate fluid. We have also lost newly metamorphosed toadlets from drowning if they are unable to exit from the water.

Trauma

Accidental death may occur occasionally in captivity, and in the wild, predation is likely to be a significant factor. The impact of the marine toad is unknown, but toads are definitely taken by mongoose, and one was recently found with a leg torn off, likely by the ameiva lizards or mongoose common on the island. Aquatic predators, such as dragonfly larvae, also prey on tadpoles.

Miscellaneous Conditions

Paralysis

In Toronto, several cases of sudden-onset posterior paralysis have been seen in PRCTs as well as other species. Affected animals show flaccid rear limb paralysis with no history of trauma. No obvious response has been seen to any particular treatment, but spontaneous recovery has occurred in some cases. Examination and electromyography indicated a neurologic syndrome associated with lower-motor-neuron dysfunction. Pathologically, axonal degeneration was seen in the lower spinal nerves. A nutritional cause, perhaps a B-vitamin deficiency, was strongly suspected, or even hypervitaminosis A.

Renal Disease

Glomerular disease was noted on a large number of necropsies. The exact cause of this disease is unclear, and it is uncertain whether a unifying etiology exists. All except one of these cases were in adult animals. Likely possibilities include glomerular disease secondary to chronic inflammatory disease, myodegeneration, or nutritional problems. Many toads with glomerulopathies had other diseases that might have predisposed them to glomerular disease, most often myopathy, dermatitis, and sepsis. Glomerulonephritis, interstitial nephritis and fibrosis, and renal cysts have been recognized in other institutions, and these are frequent pathologic findings in amphibians generally.

Spindly Leg Syndrome

In several groups of developing tadpoles, a large percentage demonstrated abnormalities of the forelimbs, the so-called spindly leg syndrome, a condition seen in other small anurans. The limbs are very small, poorly muscled, and often fail to emerge from the body. In some cases, myopathy, osteochondrosis, and spondylosis were noted, but others showed no pathologic alterations. The condition is enigmatic. Studies on tadpole and juvenile PRCTs showed no correlation with diet of the tadpoles or toadlets, although there was some correlation with adult diets, suggesting that a component in adult nutrition might be contributory.

Brown Skin Syndrome

A condition of unknown etiology has been seen in toads in Toronto. Toads with “brown skin syndrome” have dark, shiny skin on both dorsal and ventral sur-

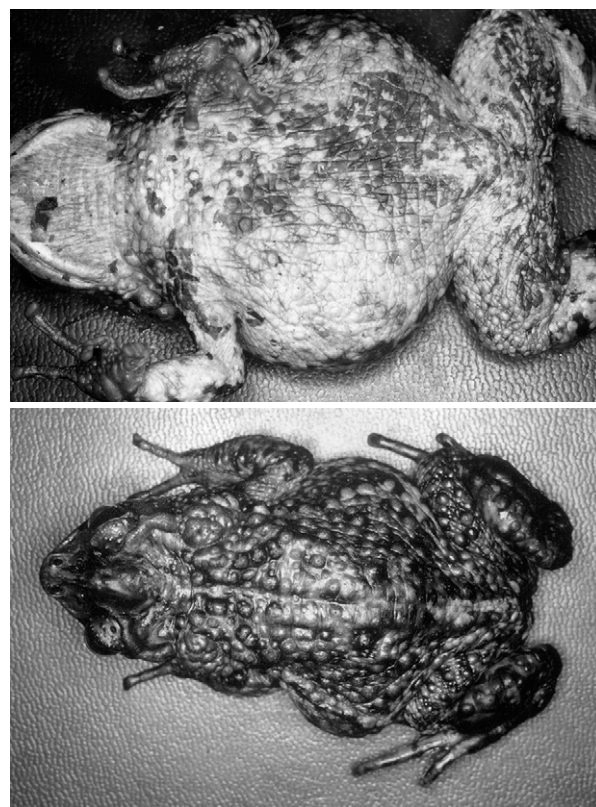


Fig 16-6 PRCT showing dysecdysis, or “brown skin disease.” Note abnormal, shiny appearance of dorsum.

faces and dysecdysis (difficult shedding) (Figure 16-6). Instead of being shed in sheets, the layer to be shed breaks up and sticks tightly to the underlying skin. A few animals have become very weak, likely because of compromised gas and fluid exchange, and several have died or faded away. Some have recovered with intensive care in high-humidity environments with oxygen and removal of dead skin. Response to soaking, antibiotics, and antifungal agents has been inconsistent. Histopathology and microbiology have been inconclusive, and samples have been negative for *B. dendrobatidis* and ranavirus DNA. Some affected toads also have shown diarrhea associated with very high numbers of intestinal protozoans. Other toads within the same tank have been unaffected, and the epidemiology does not particularly suggest an infectious process. The cause may be a result of temporary changes in nutrition that occurred months earlier.

Neoplasia

Tumors have been recognized occasionally, principally in older animals, and have included undifferentiated round-cell leukemia, a systemic myeloproliferative disease of possible granulocytic origin, lymphosarcomas, and a hepatocellular carcinoma.

Developmental Abnormalities

We occasionally see limb deformities and color mutations in groups of tadpoles. In many groups, often a small number are unusually pale in color. Most of these fail to metamorphose, but if they do, these tadpoles do not appear to thrive as well as normal, colored forms.

DIAGNOSTIC PROCEDURES

Puerto Rican crested toads have been subjected to a variety of imaging and other procedures for disease diagnosis and research purposes. Ultrasonography and plain and contrast radiography have been used in the interpretation of the “sick frog” (Figure 16-7). In a study of anatomic relationships and in an attempt to quantify ovarian development, several female toads were subjected to magnetic resonance imaging (MRI). The toads were anesthetized with MS-222 and placed in the scanner for up to 1 hour, with good differentiation of internal structures (Figure 16-8). MRI may also prove valuable for anuran diagnostic imaging.

Amphibian hematology is not used extensively for diagnosis because of the variability in values under

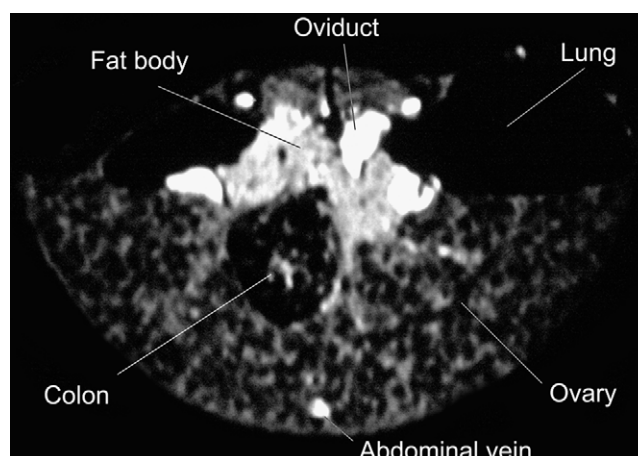


Fig 16-8 Cross-sectional magnetic resonance image of midabdomen of adult female toad showing mature ovary, oviduct, fat body, lung, and large intestine.

different physiologic conditions, the difficulty in obtaining adequate samples, and lack of data on normal values. However, a study on PRCT hematology and biochemistry has been performed (see Clinical Pathology). Collection of blood samples, best obtained from the heart, is simplified considerably with anesthesia. Without anesthesia, toads tend to inflate their bodies, making identification of the heart difficult. As with other anurans, PRCTs are averse to needles.

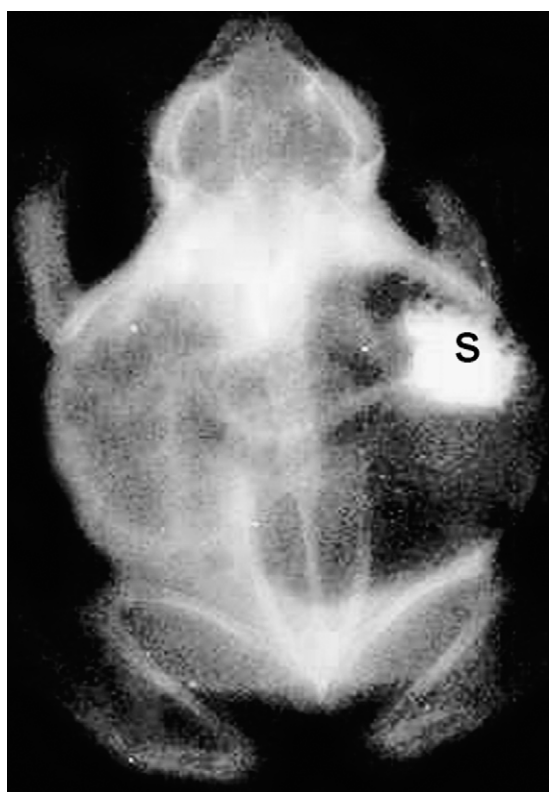


Fig 16-7 Barium x-ray film of PRCT showing lateral displacement and compression of stomach (S) by hyperinflated left lung. (Courtesy S. Ferrell.)

SURGERY

There has been little need for major surgical intervention, but PRCTs have been subjected to laparoscopic surgery for research purposes. Coelomic expansion was achieved using room air or carbon dioxide. Leg amputation through the tibia was performed following a crushing injury, and the leg healed well, although the toad eventually developed mycobacterial infection in the stump. Abdominal surgery has been performed on occasion.

CLINICAL PATHOLOGY

Blood samples may be collected and used for diagnostic purposes.^{3,9} Blood is most readily collected from the heart, and sedation greatly facilitates this process. As in other amphibians, usefulness of hematology is limited by the size and availability of samples, normal variation, and the lack of reference values for anurans. To address this deficiency, 80 8-month-old PRCTs were used in a terminal study to measure normal hematologic and biochemical values. All originated from the same batch of tadpoles and were fasted for

24 hours before blood sampling. Each toadlet was individually anesthetized in a solution of MS-222 (2 g/L) buffered with sodium bicarbonate. After exposure of the heart, blood samples were collected by cardiocentesis with a 29-gauge needle into heparinized capillary tubes. Forty samples were stained with Natt-Herrick's solution to allow manual counts of red and white blood cells using an improved Neubauer hemacytometer and light microscope. Subsamples were analyzed using an automated red blood cell counter for comparison. Differential cell counts were made after staining with Giemsa-Wright.

Table 16-1 shows the mean plasma biochemical values. Alanine transaminase (ALT) and alkaline phosphatase (ALP) showed significant gender differences: males had higher ALT values, but lower ALP values, than females. The two enzymes were also positively correlated with one another and to aspartate transaminase (AST). Males showed higher lipase values than females. No gender differences existed for total and conjugated bilirubin, and no correlation existed between creatinine and urea.

The electrolyte values, except for potassium, were maintained within a narrow range. There was a significant gender difference in chloride, sodium/potassium ratio, and potassium values. The first two parameters were higher in males than in females, whereas females had higher potassium levels. Total protein and albumin levels showed a narrow range, but significant differences existed between genders; variation in males was greater than in females.

Table 16-2 shows the blood counts and white blood cell differentials. Automated and manual erythrocyte counts were highly correlated with one another, but the manual counts were significantly higher than the automated counts. Cell differential counts vary considerably among species of amphibians. In PRCTs, lymphocytes and basophils are the predominant white blood cell types in the circulation (63% and 27%, respectively); monocytes, eosinophils, and neutrophils occur in smaller numbers. Both large and small lymphocytes and basophils may be recognized. Males had significantly larger basophil numbers and had higher leukocyte numbers overall.

Table 16-1

Plasma Biochemical Values of 40 Juvenile Puerto Rican Crested Toads

	Mean	Standard Deviation	95% CONFIDENCE LEVEL	
			Lower	Upper
Albumin (g/L)	18.0	2.7	14.0	23.0
Alkaline phosphatase (IU/L)	51.9	19.4	19.2	107.4
Alanine transaminase (IU/L)	13.7	8.0	5.0	49.5
Amylase (IU/L)	91.7	81.0	11.1	425.0
Aspartate transaminase (IU/L)	228.5	154.5	46.1	715.9
Bilirubin, total ($\mu\text{mol/L}$)	1.8	0.6	0.8	4.4
Bilirubin, conjugated ($\mu\text{mol/L}$)	0.48	0.30	0.10	1.59
Calcium (mmol/L)	2.0	0.2	1.6	2.5
Chloride (mmol/L)	76.2	6.5	63.1	89.0
Cholesterol (mmol/L)	3.8	1.9	1.6	9.2
Creatinine phosphokinase (IU/L)	2389.0	1852.0	394.7	7687.0
Creatinine ($\mu\text{mol/L}$)	28.2	5.6	18.1	43.8
Glucose (mmol/L)	2.7	1.0	1.0	5.8
Lipase (IU/L)	53.7	20.6	27.1	109.7
Phosphorus (mmol/L)	1.9	0.7	0.9	3.0
Potassium (mmol/L)	4.3	1.5	1.3	7.1
Protein, total (g/L)	38.5	5.5	28.0	51.0
Sodium (mmol/L)	109.5	5.9	96.2	121.0
Urea (mmol/L)	15.0	6.5	4.4	20.9
Sodium/potassium ratio	30.4	15.9	15.0	81.9
Blood volume (mL)	0.75	0.31	0.35	1.63
Serum (mL)	0.41	0.18	0.10	0.89
Weight (g)	14.0	2.6	9.4	20.9

Table 16-2

Blood Counts and White Blood Cell Differentials of 40 Puerto Rican Crested Toads

	Mean	Standard Deviation	95% CONFIDENCE LEVEL	
			Lower	Upper
Automated erythrocyte count ($\times 10^{12}/L$)	0.99	0.11	0.72	1.24
Manual erythrocyte count ($\times 10^{12}/L$)	1.24	0.21	0.81	1.63
Manual white blood cell count ($\times 10^9/L$)	10.16	3.07	5.28	16.78
Neutrophil percentage (%)	4.4	2.5	0.03	12.9
Lymphocyte percentage (%)	63.8	8.2	41.2	79.0
Basophil percentage (%)	27.7	7.2	15.0	43.0
Eosinophil percentage (%)	3.0	3.2	0	14.9
Monocyte percentage (%)	1.3	1.4	0	6.0
Blood volume (mL)	0.90	0.22	0.45	1.60
Hematocrit (%)	32.9	3.7	24.1	41.0
Hemoglobin (g/L)	105.2	13.9	80.2	134.9
Weight (g)	12.8	2.6	8.0	20.0

DISEASE CONTROL AND PRE-RELEASE SCREENING

Infectious diseases are being increasingly recognized as threats to wildlife, and several could be potentially devastating if introduced to naive populations.⁴ Chytridiomycosis and ranavirus have emerged as major threats to the survival of wild amphibian populations. PRCTs in the SSP and the release program in Toronto are maintained in a facility with minimal contact with other amphibians. Animals destined for release should ideally be held in permanent quarantine separate from other amphibians and must be subjected to ongoing health monitoring.

Before stressful events such as prebreeding environmental manipulation or shipment to other institutions, prophylactic treatment with itraconazole (Sporanox, Ortho-McNeil, Raritan, NJ) or enilconazole (Imaverol, Merial, Baie d'Urfe, QC, Canada) for superficial fungal infections, including chytrids, is indicated. Toads are soaked in a depth of 2 cm of 0.01% itraconazole in 0.6% saline for 5 minutes daily for 5 days before cooling. At the end of the cooling period and before placing the toads into the breeding tank, 0.01% itraconazole is delivered as a rinse spray using a hand-held spray bottle.

Antibiotics are used if breeding animals show evidence of sepsis or cutaneous lesions. For PRCTs to qualify for release, there must have been (1) no deaths in the release group in the previous 30 days or since hatch, (2) no unknown diagnoses of contagious disease as a cause of death of in-contact animals for the prior 60 days, and (3) no medical treatments of the release

group in the previous 60 days. The occurrence of an undiagnosed skin disease in Toronto toads, even though there was no epidemiologic evidence of a contagious or infectious etiology, rendered it expeditious to cease temporarily the breeding and release of toads from this group.

To limit potential risks in releasing a captive amphibian, health criteria need to be considered, and disease screening is mandatory. It has been postulated that the widespread dissemination of chytridiomycosis around the world was caused by the worldwide trade in frogs for laboratory and food purposes. Pre-release screening of PRCTs for pathogens is problematic because tadpoles are released at less than 2 weeks of age to integrate them into the wild population at the earliest life stage so that they will return to the natal pond to breed. Subsamples of developing tadpoles are submitted for histologic examination of mouthparts for chytrids before release of the main cohort; to date, all have been negative. The use of a rapid-detection PCR assay may be valuable in the future.²

The risk of introducing disease into wild populations is mitigated by isolation and monitoring of the captive population. PRCTs are released as tadpoles into remote, purpose-built ponds to encourage expansion of the range while avoiding a negative impact on the existing population.

Acknowledgments

Both I and Puerto Rican crested toads everywhere are indebted to Bob Johnson, whose tireless efforts

have helped ensure a future for the species, and to Andrew Lentini, Dianne Devison, and the others who have cared for and nurtured many thousands of toads. I am also grateful to the veterinarians, keepers, and curators who participate in this program and have contributed their knowledge and experiences, and to Stephen Barabas for undertaking the hematologic study.

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Amphibian Chytridiomycosis

ALLAN P. PESSIER

Chytridiomycosis is an important emerging disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd). Although previously classified within the kingdom Protista, organisms in the phylum Chytridiomycota (“chytrids”) are now known to be true fungi that produce characteristic, motile, posteriorly uniflagellate zoospores within discrete fungal bodies termed *thalli*. Chytrids are ubiquitous in aquatic environments, and although several species are known pathogens of plants, fungi, or invertebrates, none were reported to infect vertebrates until the description of Bd in amphibians.^{2,21} To date, only Bd has been associated with amphibian disease, and Koch’s postulates have been fulfilled.²⁷

Preliminary reports of chytridiomycosis in the late 1990s described Bd as either an aquatic, fungal-like protist or a *Perkinsus*-like protozoan. In addition, some reports of infection with *Basidiobolus ranarum* in toads were subsequently determined to have been caused instead by infection with Bd.^{24,31} Infection has now been recognized in a wide range of both anuran (frogs and toads) and urodele (salamanders and newts) amphibians. A report of infection in the order Gymnophiona (caecilians) is anecdotal and needs to be confirmed.²⁵

Although a significant disease problem in both captive and free-ranging animals, chytridiomycosis is most important because of an association with mass mortality events and population declines in the United States, Europe, Latin America, and Australia.^{2,5,7,12,24} Concerns about the effects of chytridiomycosis on free-ranging populations, including the possibility of disease-associated extinctions, have resulted in calls for ex situ species salvage and breeding programs that may significantly impact zoologic collections.

CHYTRIDIOMYCOSIS AND AMPHIBIAN POPULATION DECLINES

Amphibian population declines have been increasingly recognized since the late 1980s and have generated much public and scientific interest. A recent survey

suggests that 32.5% of known amphibian species are globally threatened and 43.2% are experiencing a population decrease.³⁸ Although in some declines a clear anthropogenic cause such as habitat loss or species exploitation may be implicated, in many others the cause is not obvious (“enigmatic decline”). Of the enigmatic-type population declines, chytridiomycosis and climate change are most frequently cited as potential causes.

Population declines attributed to chytridiomycosis are best documented in stream-dwelling species at high elevations in the rain forests of Central America and eastern Australia.^{19,41} In both locations, evidence suggests temporal and geographic progression of declines.⁸ The apparent southward progression of disease incidence in Costa Rica and Panama has allowed for prediction of future sites of decline.²⁰ Disease progression may have significant implications for worldwide amphibian species diversity because up to half of all species live in neotropical regions that environmental modeling suggests could be a suitable niche for Bd.³⁷

It is unclear whether Bd is a novel pathogen that has recently been introduced to naive populations or an endemic pathogen that has emerged because of environmental or other cofactors.³⁵ It may be that both circumstances exist, depending on geographic location. Preliminary genetic information obtained from isolates originating from several continents indicates that Bd is a recently emerged clone, possibly consistent with an introduced novel pathogen.²³ Research on the means of introduction of Bd to new locations has focused on international movement of amphibians for food, laboratory research, and the pet trade. In particular, the African clawed frog (*Xenopus laevis*) and the bullfrog (*Rana catesbeiana*) are species that have been widely moved or introduced worldwide and are good potential reservoir hosts for Bd because they carry infection without significant clinical signs.^{9,40} The occurrence of Bd-associated mortality events at lower temperatures (within preferred temperature ranges for Bd) suggests that environmental cofactors may play a role in mortality events resulting from either recent

introduction of Bd to a region or exacerbation of endemic infections.^{3,33} Stable endemic Bd infection of populations has been documented after catastrophic declines presumed to have resulted from introduced Bd infection,³⁶ as well as in populations without documented declines.²⁸

PATHOLOGY AND PATHOGENESIS

Lesions of chytridiomycosis are limited to keratinizing epithelium in the skin of postmetamorphic animals and the mouthparts (tooth rows and jaw sheaths) of tadpoles.^{2,32,34} Dissemination to deeper portions of the skin or to viscera does not occur. Lesions consist of varying degrees of epidermal hyperplasia and hyperkeratosis, with intralesional thalli characteristic of Bd (Figure 17-1). The keratinized layers (stratum corneum) of amphibian skin are usually very thin, and hyperkeratosis may be overlooked if using criteria established for other species. Associated inflammatory cell infiltrates are an inconsistent finding and, when observed, are usually in association with severe or chronic infections or in cases with secondary bacterial or fungal infection. Secondary infections are common, presumably because environmental bacteria and fungi become trapped in excessive keratin layers or within empty thalli of Bd that have expelled zoospores. The severity and distribution of lesions may range from relatively minimal focal lesions in subclinically infected animals¹³ to severe, multifocally extensive to diffuse lesions considered to be clinically significant.

Characteristic features of Bd thalli aid in identification in cytologic preparations or histologic section. The spherical thalli, which are intracellular in superficial

keratinocytes, range from approximately 7 to 20 μm in diameter, and mature thalli (zoosporangia) may contain discrete, 1- to 2- μm basophilic zoospores. Empty thalli that have discharged their zoospores are common and should be distinguished from cross sections of fungal hyphae or ducts of cutaneous glands. Flask-shaped thalli with prominent discharge papillae (discharge tubes) can usually be found in most heavy infections (Figure 17-2). Colonial thalli have evidence of fine internal septation and are best appreciated in empty thalli or in Gomori's methenamine silver (GMS)-stained sections. An inconsistent, but sometimes helpful, finding in GMS-stained sections are thin, rootlike extensions from thalli termed *rhizoids* (Figure 17-2, D).

The mechanism by which Bd causes death of susceptible animals is still unclear. Disruption of normal skin function, especially in regard to water absorption and electrolyte balance, and secretion of a mycotoxin are the most frequently cited hypotheses. Some clinically affected animals show evidence of dehydration and hemoconcentration, which may provide support for disruption of skin function.¹¹ Death in *Bufo boreas* tadpoles shortly after exposure to cultures of Bd might support the role of a mycotoxin.⁴ There is evidence for both species-related and age-related differences in susceptibility to clinically significant infections.^{10,13,18}

Transmission of Bd infection is through the motile, flagellated zoospores and may occur by direct contact between animals or by contact with water or substrates used in housing affected animals. Extension of infection from the keratinized mouthparts of tadpoles may occur as the tadpoles metamorphose and develop a keratinized epidermis.^{18,34}

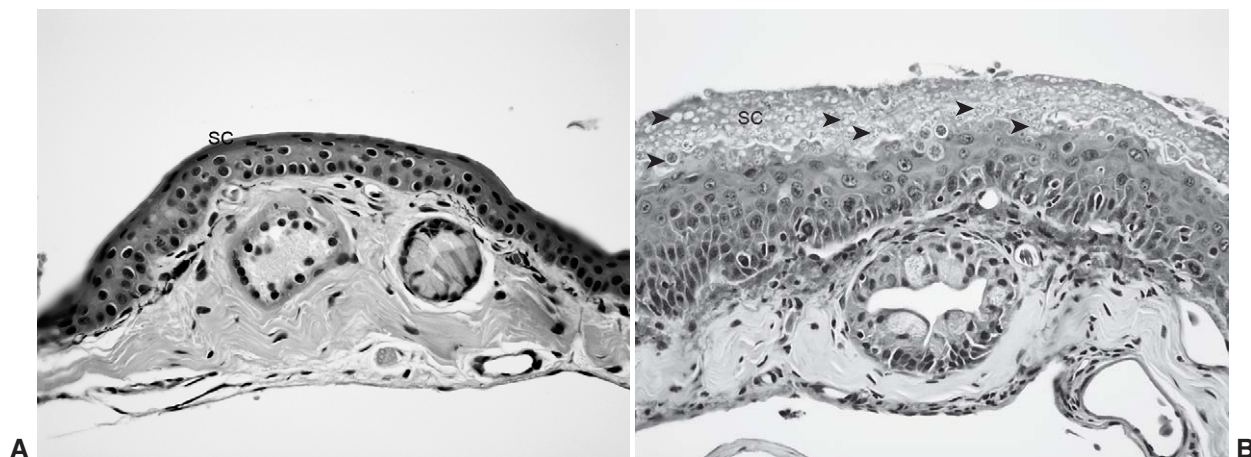


Fig 17-1 **A**, Histologic section of normal skin from boreal toad (*Bufo boreas boreas*). The stratum corneum (SC) is thin and only one or two cell layers thick. **B**, Histologic section of skin from Wyoming toad (*Bufo baxteri*) with severe chytridiomycosis. There is moderate epidermal hyperplasia, and the SC is greatly thickened with numerous chytrid thalli (arrowheads). (See Color Plate 17-1.)

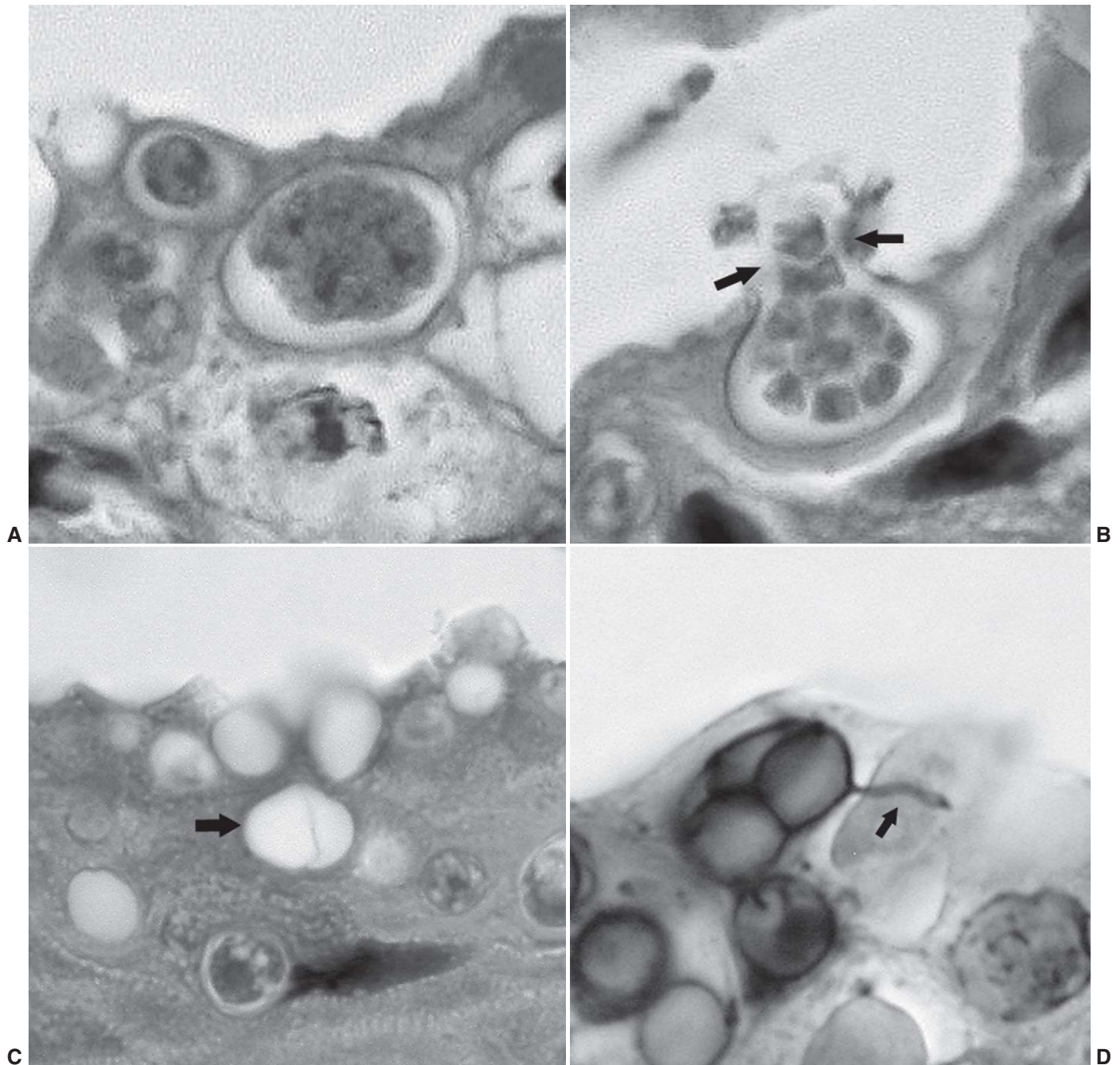


Fig 17-2 **A**, Four immature, spherical chytrid thalli in stratum corneum of a boreal toad (*Bufo boreas boreas*). **B**, Mature chytrid thallus (*B. boreas boreas*). Also visible in this section is the discharge tube (arrows), giving the thallus a flasklike shape. **C**, Numerous empty chytrid thalli that previously discharged zoospores in the keratinized mouthparts of mountain stream tadpole (*Ptychohyala* sp.). The thallus in the center (arrow) has faintly visible internal septation (colonial thallus). **D**, Gomori's methenamine silver (GMS)-stained section showing rootlike extension (rhizoid, arrow) from a colonial thallus in the stratum corneum of a Limon giant glass frog (*Centrolenella ilex*). (See Color Plate 17-2.)

CLINICAL SIGNS

Clinical signs of chytridiomycosis are variable and range from unexpected death without premonitory signs to animals with evidence of significant skin disease. The most common cutaneous signs of chytrid-

iomycosis are excessive shedding ("sloughing") of skin, rough or granular changes in skin texture, and brown to red (hyperemia) skin discoloration. Findings are most often distributed on the ventral body and feet of terrestrial animals, but may be diffuse in totally aquatic species. Hyperemia and other features, such as

cutaneous ulceration, are more common in animals with secondary bacterial, fungal, or water mold (Oomycetes) infections. In these cases, chytridiomycosis should be distinguished from other potential causes of “red leg” syndrome, including bacterial septicemia and iridovirus infection.

Other clinical signs that may be observed include postural changes, in which animals hold their legs away from the body, apparently to avoid contact with substrate; avoidance of or increased preference for water; anorexia; lethargy; and neurologic signs such as loss of righting reflex.

Tadpoles infected with Bd are usually asymptomatic and may serve as reservoirs of infection for post-metamorphic animals. Detailed gross examination of the keratinized mouthparts may show areas of depigmentation, which should be distinguished from other potential causes of mouthpart abnormality, including chemical exposure and low environmental temperature.³⁴ Reduced body mass of infected tadpoles and deaths of some individuals in acute-exposure studies have been reported for selected species.^{4,30}

DIAGNOSIS

Diagnosis of infection with Bd has been described using both morphologic (cytology and histopathology) and molecular techniques (polymerase chain reaction). Routine fungal culture is not helpful because Bd requires specialized techniques for isolation.²¹ Selection of a diagnostic method depends on the purpose of the investigation. For example, surveys of wild populations for the presence, absence, or prevalence of Bd infection increasingly rely on molecular methods, whereas investigators of mortality events prefer histopathology, which allows assessment of the clinical significance of lesions (and may exclude other disease entities).

Diagnosis using cytology or histopathology is by demonstration of characteristic thalli of Bd within the cytoplasm of keratinocytes (see Pathology and Pathogenesis). For rapid diagnosis of heavy Bd infection in a clinical setting, cytology or wet mounts may be useful. These are not appropriately sensitive techniques for screening wild populations or animals in quarantine and may not detect all clinical cases. Samples are obtained by recovering shed skin fragments or by gentle skin scraping using a toothpick or the end of a cotton-tipped applicator. Samples are air-dried on slides, stained using a rapid hematologic dye, and examined under oil immersion.^{27,31} Diagnosis should only be made if unequivocal Bd thalli are

observed, because yeasts and fragments of Oomycetes water molds may occasionally be confused for degenerate thalli.

Diagnosis by histopathology may be made from both clinical and necropsy samples. Immunohistochemistry using polyclonal antibodies has been described as an aid in histologic diagnosis; for most clinically significant infections, however, diagnosis based on morphologic criteria alone will be sufficient.³⁹ A potentially useful and noninvasive clinical sample is to fix fragments of shedding (sloughing) skin in formalin for histologic examination. This technique occasionally provides better morphologic detail of Bd thalli (especially empty thalli) than that obtained by cytologic examination of skin fragments. Histologic examination of toe clips has been described for wild frogs, but this technique may be too invasive for clinical utility. At necropsy, routine collection of multiple skin sections for histopathology, including the ventral pelvic (“drink patch”) region, ventral legs, and feet, is suggested both for surveillance of amphibian collections and for diagnosis of suspected clinical cases. For very small animals such as dendrobatids, this may be accomplished by processing multiple whole-body sections (including feet) after decalcification.

Detection of Bd deoxyribonucleic acid (DNA) in skin swabs or scrapings by either conventional or real-time Taqman polymerase chain reaction (PCR) has been a recent technical advance.^{1,6} These methods are especially helpful for determining incidence and prevalence of infection in wild populations, screening of animals both in quarantine and before release to the wild from captive breeding programs, and for clinical diagnostics. Although the PCR methods are extraordinarily sensitive, early or subclinical Bd infections may be unevenly distributed, and empty thalli (no longer containing DNA) are common; thus the possibility of false-negative results from sampling should be considered on interpretation. Ideally, swabs or scrapings submitted for PCR should include material from more than one location on the ventral body and feet. In the United States, PCR for Bd is commercially available through Pisces Molecular, Boulder, Colorado (303-546-9300; jwood@pisces-molecular.com).

DISEASE CONTROL AND TREATMENT

Control of chytridiomycosis in amphibian colonies includes disinfection of enclosures and implementation of husbandry practices that prevent cross-contamination of enclosures with water or substrate material. The infectious zoospores are sensitive to desiccation,

and to date, no resting stages resistant to drying have been identified. Therefore the primary means of transmission in animal groups is by direct animal-to-animal contact or contact with water or moist substrates used in housing infected animals. Bd may persist for several weeks in tap water and sterilized lake water, for several months in sterilized moist river sand, and up to 3 hours on avian feathers.^{15,16} Most of the common disinfectants, including quaternary ammonium compounds and sodium hypochlorite (bleach), are effective in killing Bd, as is heat (47° C for 30 minutes). However, ultraviolet (UV) light is relatively ineffective.¹⁷

Treatment for chytridiomycosis has been described using nonspecific chemical agents, specific antifungal agents, and elevation of environmental temperature. Topical treatment such as immersion baths, rather than systemic treatment, is suggested because of the superficial cutaneous location of the infection. If there is no prior experience using a specific drug in a particular amphibian species or age group, consideration could be given to treatment of sentinel animals before treatment of large groups. For individual animals with severe disease, supportive care may be helpful, including fluid therapy for dehydration and antibiotics to control secondary bacterial infections. Treatment has been successful both for individual animals and for amphibian colonies, although concerns exist that treatment alone cannot guarantee clearance of Bd from all groups of frogs.¹⁴ Experimental studies on the clearance of Bd after treatment, as well as studies examining combinations of treatments (e.g., antifungal baths and increased environmental temperature), are warranted.

Treatment using a bath of 0.01% itraconazole has been described experimentally^{18,26} and subsequently applied to a variety of species in zoologic institutions and conservation programs. The bath is prepared by diluting the commercially available 1% itraconazole solution (or a compounded suspension) in 0.6% saline or amphibian Ringer's solution.⁴³ Animals are placed in a shallow bath for 5 minutes daily for up to 11 days. The bath should be periodically agitated to ensure that the entire skin surface is treated. Itraconazole should be avoided in tadpoles and used with caution in very young, postmetamorphic animals because treatment-associated deaths have been observed in both Australia and the United States. It is unknown whether these deaths are attributable to the drug itself or to other components of the itraconazole formulation. Use of fluconazole in tadpoles did not appear to have toxic effects.²²

Treatment of chytridiomycosis in African clawed frogs (*Xenopus tropicalis*) was accomplished using a

solution of 25 ppm formalin and 0.1 mg/L malachite green for 24 hours, repeated every other day for a total of four treatments.²⁹ This treatment could be considered for use in other aquatic species as long as potential adverse effects of both formalin and malachite green are taken into account.⁴³

The preference of Bd for lower temperatures (optimum growth at 17°-25° C)³³ has the potential to be exploited as a primary or adjunctive treatment in species that may tolerate higher environmental temperatures. Animals held at 37° C for less than 16 hours appeared to be cleared of Bd infection,⁴² and experimental animals housed at 27° C had apparent elimination of infection by 98 days after onset.³ Use of elevated temperatures in the treatment of naturally occurring infections has not been reported.

Treatment of entire groups of captive animals may be considered because of species salvage efforts that bring infected wild animals into captivity, or because of repeated outbreaks in closed colonies that presumably occur because of subclinically infected carrier animals within a group. In my experience, two separate captive breeding groups of Wyoming toads (*Bufo baxteri*), had repeated outbreaks occurring over several years, and chytridiomycosis was the major cause of mortality. Colony-wide treatment using a combination of itraconazole baths and disinfection of enclosures was instituted, and no chytridiomycosis-associated deaths were documented for the next 3 years using aggressive clinical and necropsy surveillance.

QUARANTINE AND REINTRODUCTION PROGRAMS

General considerations for amphibian quarantine have been described and are available online (see later listing). For amphibians entering into quarantine, an initial consideration is the health history of the originating collection, which ideally would include thorough clinical and necropsy surveillance for infectious diseases. Such surveillance data are usually not available for animals obtained from dealers or acquired from the wild. Complete necropsies, including histopathology, should be performed on all animals that die in quarantine and may provide a comprehensive measure of health status for animals destined to enter an animal collection. Histopathology for chytridiomycosis should include multiple skin sections, as noted previously, and may be helpful even in autolyzed specimens. If available, incoming amphibians (or subsets of incoming animals in large groups) may be screened for Bd by PCR of skin scrapings or skin

swabs. Prophylactic antifungal treatment of entire groups may be considered if animals are coming from high-risk situations or if Bd-positive animals are identified by PCR or histopathology.

Similar measures are suggested for prevention and control of Bd infections in animals involved in captive breeding and reintroduction programs. Ideally, breeding groups of amphibians producing offspring for later release into the wild should be maintained in a state of permanent quarantine to minimize the risk of introduced infectious agents. Health surveillance of breeding groups to include necropsy and histopathology of all animals that die should be part of the standard operating procedures. If chytridiomycosis is identified, prophylactic group treatment may be initiated, followed by PCR and necropsy surveillance of the group for any subsequent infections. Animals from groups with identified Bd infections should not be used for releases to the wild until long-term (at least 1 year) group surveillance suggests that infection has been eliminated. Screening by PCR of a subset of animals destined for release is an additional option, but this may not always be economically feasible because of the large numbers of animals involved in amphibian release programs. A significant concern for many release programs is not Bd infection in animals destined for release, but rather preexisting Bd infection in free-ranging animals already present at release sites.

Amphibian Disease Website

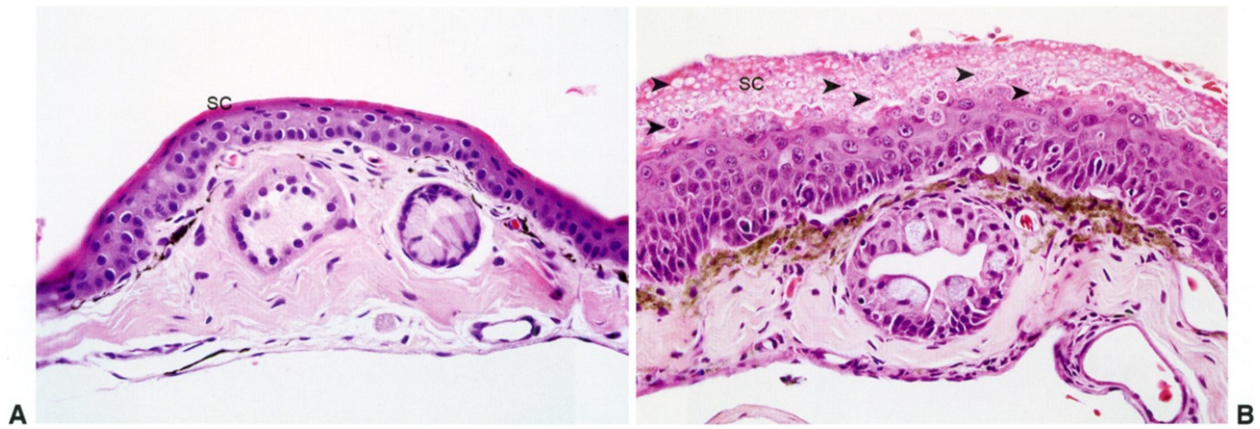
www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm.

The Amphibian Diseases Home Page is a useful website with information on chytridiomycosis, including published articles, diagnostic and quarantine protocols, and a comprehensive bibliography of amphibian diseases.

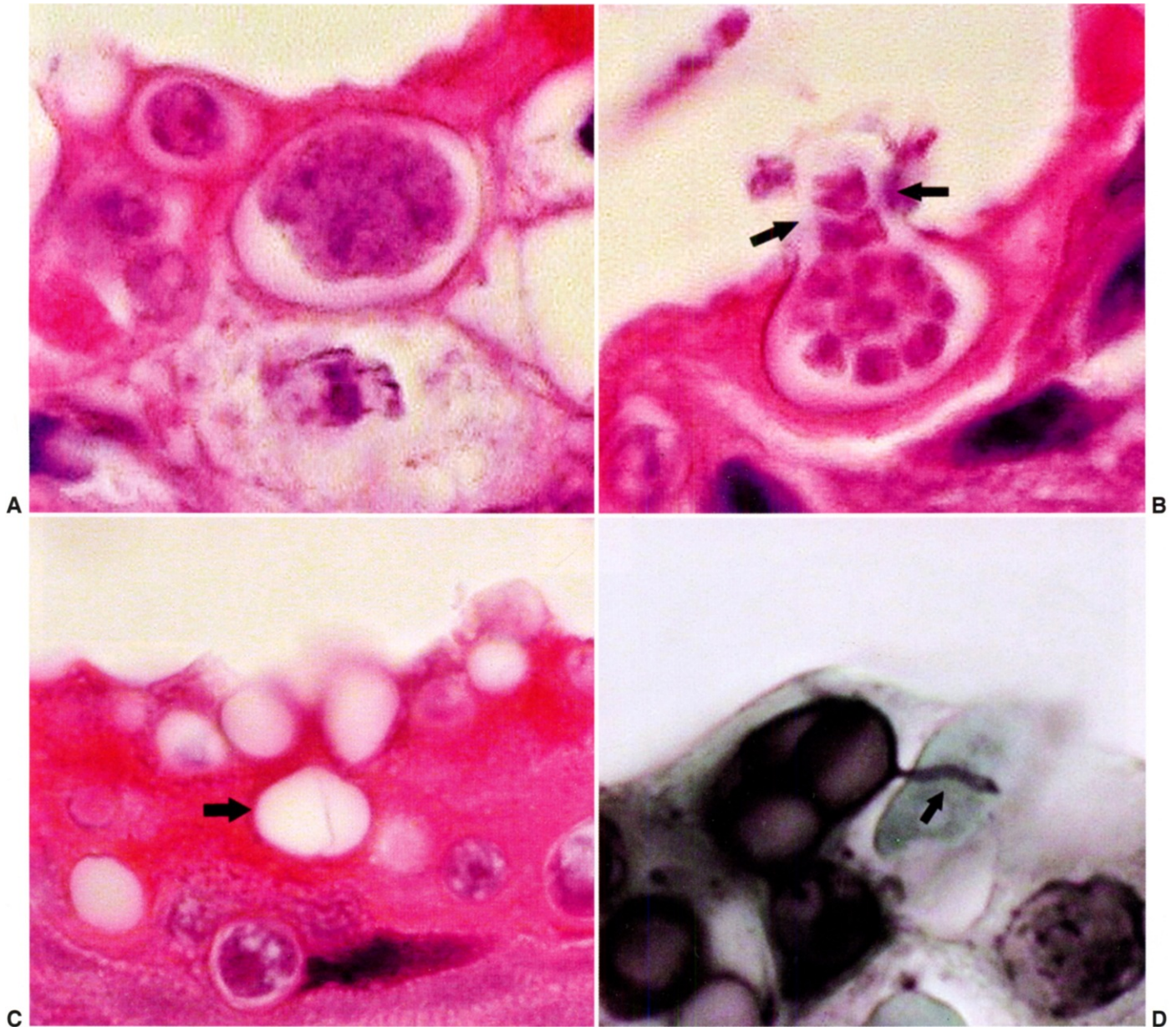
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Color Plate 17-1 **A**, Histologic section of normal skin from boreal toad (*Bufo boreas boreas*). The stratum corneum (SC) is thin and only one or two cell layers thick. **B**, Histologic section of skin from Wyoming toad (*Bufo baxteri*) with severe chytridiomycosis. There is moderate epidermal hyperplasia, and the SC is greatly thickened with numerous chytrid thalli (arrowheads). (For text mention, see Chapter 17, p. 138.)



Color Plate 17-2 **A**, Four immature, spherical chytrid thalli in stratum corneum of a boreal toad (*Bufo boreas boreas*). **B**, Mature chytrid thallus (*B. boreas boreas*). Also visible in this section is the discharge tube (arrows), giving the thallus a flasklike shape. **C**, Numerous empty chytrid thalli that previously discharged zoospores in the keratinized mouthparts of mountain stream tadpole (*Ptychohyla* sp.). The thallus in the center (arrow) has faintly visible internal septation (colonial thallus). **D**, Gomori's methenamine silver (GMS)-stained section showing rootlike extension (rhizoid, arrow) from a colonial thallus in the stratum corneum of a Limon giant glass frog (*Centrolenella ilex*). (For text mention, see Chapter 17, p. 139.)

CHAPTER 18

Raising Giant Tortoises

JEAN-MICHEL HATT

Only a few institutions have been successful in regularly breeding and raising giant tortoises. Knowledge of tortoise management and breeding remains scarce, and minimal scientific data exist. The published peer-reviewed data on the topic largely have been generated from Galápagos tortoises at one facility (Zurich Zoo, Switzerland). Although the author has taken great care to collect scientific data from as many sources as possible, large parts of this chapter still reflect personal observations. It is hoped that in the future this information will be subjected to scientific analysis to allow a transition from experience-based to evidence-based raising of giant tortoises.

The order tortoises Testudines include the families Testudinae and Emydidae. The Testudinae comprise 14 genera. From fossils it is known that several Testudinae existed as giant forms on all continents except Australia and Antarctica. At present, giant tortoises have survived on Aldabra, the Seychelles, and the Galápagos Islands. The taxonomy of the surviving genera is still under debate; Table 18-1 summarizes the different nomenclature. This chapter uses the terms *Geochelone nigra* and *Geochelone gigantea* for Galápagos tortoises and Seychelles tortoises, respectively.

The current number of Galápagos tortoises is estimated at 12,000 to 15,000²³ and the Seychelles tortoises at 100,000.¹ All giant tortoises are listed by the Convention on International Trade in Endangered Species of Wild Fauna and Flora⁵; Seychelles tortoises are classified under Appendix II and Galápagos tortoises under Appendix I.

The major threat to giant tortoises is feeding concurrence by introduced domestic animals, especially goats, dogs, and pigs, as well as predation by rats. In addition, illegal trade still has a significant negative impact on population densities, especially in the Seychelles tortoises. In 2002, fewer populations of Galápagos tortoises were affected by food concurrence. Wild pigs and dogs on Santiago and Isabella Islands have been eradicated. The islands of Pinta Española

and Santa Fe no longer have goats. Goats are still present on Isabela, Santiago, and San Cristóbal.¹⁸ Unfortunately, Galápagos tortoises face a new threat, which is habitat reduction. The archipelago prospers and has the highest overall standards of living of any province in Ecuador. This has caused massive increases of the human population on the islands. In an area where 95% of the land is a national park, this leads to conflicts.

Considering that the survival of giant tortoises is far from secure, captive breeding is critical. Breeding programs exist both on the Galápagos Islands (Charles Darwin Research Station) and on the Seychelles (Seychelles Giant Tortoise Conservation Project). Breeding outside these islands is of importance not only to eliminate exportation of wild animals, but also for educational and scientific purposes.

UNIQUE ANATOMY

The two species of giant tortoises may readily be differentiated by the form of their head and the carapace (Table 18-2). Adult giant tortoises may grow to a straight carapace length (sCPL) of more than 130 cm and a body weight of 300 kg. Seychelles tortoises are approximately 20% smaller and less heavy than Galápagos tortoises.

The general anatomy of giant tortoises is comparable to that of Testudines in general. Of special interest for breeding is gender differentiation. Adult animals may readily be differentiated externally based on plastron and tail. As in many other tortoises, adult males have distinctly longer tails than females, and the plastron is concave. Determining gender in hatchlings and juvenile giant tortoises, however, is not readily possible. One way to differentiate gender in Seychelles tortoises may be the number of tail scales. As the tail grows, the scales elongate, but new tail scales are not formed. Female Seychelles tortoises were found to have 8 to 11 scales, whereas males have 12 to 14 scales.¹⁰

Table 18-1

Taxonomy of Giant Tortoises

	SEYCHELLES TORTOISES		GALÁPAGOS TORTOISES	
	(CITES, 2005)	(Gerlach, 2004)	(CITES, 2005)	(Pritchard, 1996)
Order			Testudines	
Family			Testudinae	
Genus	<i>Geochelone</i>	<i>Dipsoschelys</i>		<i>Geochelone</i>
Taxon	<i>Geochelone gigantea</i> (three subspecies)	Six species	<i>Geochelone nigra</i>	<i>Geochelone nigra</i> (10 subspecies)

Table 18-2

Anatomic Differences between Galápagos (*Geochelone nigra*) and Seychelles (*Geochelone gigantea*) Tortoises

	<i>G. gigantea</i>	<i>G. nigra</i>
Head	Head with similar diameter as neck Rounded ridge of nose, pointed nose	Head wider than neck Short nose
Prefrontal scales	Large	Small
Nose	Vertical, slitlike opening Soft tissue flap on the nasal septum allows animal to close off the nasal cavity proper; drinking through nose possible.	Round opening No drinking through nose possible
Nuchal scute	Present in 98%	Absent
Caudal scute	Mostly double	Single

Modified from Ebersbach K: Doctoral thesis, Hannover, Germany, 2001, University of Hannover.

This method has not been investigated in Galápagos tortoises.

Endoscopic gender differentiation has successfully been performed in Galápagos tortoises ages 12 months and older. The ovary with primary follicles or the inactive testicle may readily be visualized (Figures 18-1 and 18-2). Access with a 2.7-mm rigid endoscope, with a 30-degree distal lens offset, is from the left prefemoral region, as in other chelonians. Carbon dioxide is used for insufflation.

CAPTIVE MANAGEMENT OF GIANT TORTOISES

Because of their large body size, captive management of giant tortoises may be a challenge outside their natural climatic condition. The following describes the enclosure of Galápagos tortoises at Zurich Zoo, where successful breeding has occurred.¹⁵ Three adult and four juvenile animals are kept in a combined indoor (65 m²) outdoor (400 m²) enclosure. The outdoor enclo-

sure has a 12-m² shelter that offers constant temperatures of 27° C. The animals remain on the outside enclosure as long as night temperatures do not fall below 15° C.

The indoor enclosure has a 30-m² heated surface (24° C). For oviposition a special nesting area has been prepared that measures 2 × 3 m. The depth is 50 cm, and it is filled with sand. This area is under 24-hour lapse-time video supervision during months when oviposition is expected. This allows safe removal of the clutch for artificial incubation. Special attention is given to the photoenvironment of the indoor space. A solarium with infrared (IR) and ultraviolet (UV) light offers a local hot spot of 38° C twice daily for 30 minutes, usually after feeding. Special high-intensity mercury light sources are used, with the aim to expose the animals to full-spectrum light. By definition, "full spectrum" means a color temperature of 5500° K, a color-rendering index of 90 and above, and a spectral power distribution for UV as well as visible light similar to that of open-sky, natural light at noon. Relative humidity in the inside enclosure is 60% to

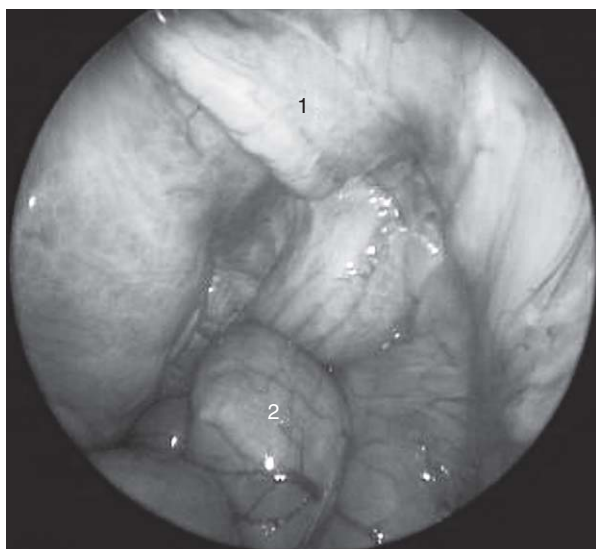


Fig 18-1 Coelioscopy in 6-year-old Galápagos tortoise (*Geochelone nigra*). 1, Juvenile testicle; 2, intestine. (See Color Plate 18-1.)

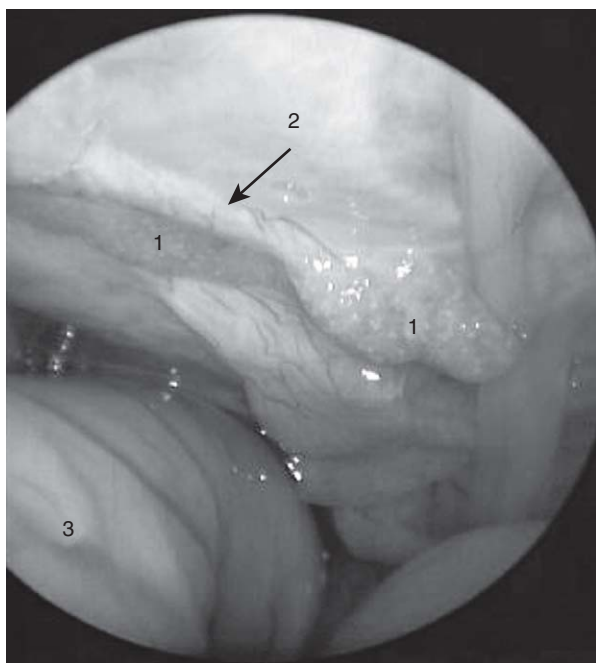


Fig 18-2 Coelioscopy in 4-year-old Galápagos tortoise (*Geochelone nigra*). 1, Juvenile ovary with primary follicles; 2, oviduct; 3, intestine. (See Color Plate 18-2.)

70%. Both inside and outdoor enclosures have a shallow pool, often used by giant tortoises. A mud wallow is not available but would be an important addition because giant tortoises readily use wallows. In our experience a mud wallow in the outside enclosure has the disadvantage that animals remain in the mud when temperatures drop, and it is then difficult to remove an adult giant tortoise without the risk of causing damage to the carapace.

NUTRITION

Numerous adult Seychelles and Galápagos tortoises are kept in many zoos and institutions around the world. Despite this, surprisingly little is known about the exact nutritional needs of these animals. Even less is known of the requirements of juvenile giant tortoises. Possible nutrition-related disorders are (excessive) geophagy with subsequent constipation, loose stools, bloat, and metabolic bone disease.

An evaluation questionnaire found that zoos keep Seychelles tortoises mainly on domestic fruits and vegetables, or grass, hay, and other roughage. Such a diet is not adequate. Giant tortoises are herbivorous, and their natural diet consists of grass, leaves, flowers, and fruits. In the wild, giant tortoises consume what is described as “tortoise turf,” a complex of grasses, sedges, and herbs.⁸ In Seychelles tortoises an apparent dry-matter digestibility of 30% was measured for the turf. Carnivory and coprophagia have been observed but do not make up more than 0.5% of the total ingested diet.

Seychelles tortoises appear to be more of a grazer, in contrast to Galápagos tortoises, which are more of a browser. To reach higher leaves and flowers, animals will climb on top of each other. Additionally, browsing species develop a saddle-shaped carapace that allows better vertical extension of the head, compared with the dome-shaped carapace.

Giant tortoises are well adapted to increase their growth rate during vegetation periods. In their natural habitat, giant tortoises lose significant amounts of weight during the dry season, in contrast to the vegetation period, when body fat depots are produced. During dry periods, giant tortoises increase digestibility of the diet by increasing intestinal transit time by a factor of five. A longer transit time allows the gut flora more time for fermentation, which increases digestibility.

The diet of juvenile giant tortoises at Zurich Zoo consists of 90% chopped high-quality grass hay. To this a variety of herbs, dried leaves, and occasionally, produce are added. The amount of fresh food offered to hatchlings and juveniles up to age 4 years varies between 3% and 5% of their body weight per day.

Giant tortoises are well adapted to a diet rich in fiber. Four captive-bred juvenile Galápagos tortoises ages 4 and 5 years were fed a controlled diet for 32 days. The diet consisted of 77% hay, 15% tortoise pellets, and 8% apples on a dry-matter basis. Diet analysis revealed 95.7% organic matter, 11.3% crude protein, 20.5% crude fiber, 22.6% acid detergent fiber, 5.0% acid detergent lignin, and 17.6% cellulose. Based on total fecal collection during 7 days, the

following average dry-matter digestibilities were calculated: 65% for dry matter, 67% for organic matter, 63% for crude protein, 55% for crude fiber, 49% for acid detergent fiber, 41% for acid detergent lignin, and 54% for cellulose. Compared with mammalian hindgut-fermenting herbivore species (domestic horses, Asian elephants, Indian rhinoceroses) on a diet of hay and concentrates, the juvenile Galápagos tortoises showed a digestion of similar efficiency. An increase in crude fiber content resulted in a reduced digestibility. If a reduction in dietary digestibility is to be achieved in juvenile Galápagos tortoises, crude fiber levels of 30% to 40% on a dry-matter basis should be the target.¹³

Adequate calcium (Ca) supplementation is critical for the healthy development of giant tortoises. Oyster shell powder and calcium carbonate has successfully been added to the diet. In juvenile Galápagos tortoises, we found that an increased Ca-to-phosphorus (P) ratio did not result in a reduced Ca uptake.¹⁶ Apparent Ca digestibility at Ca/P levels of 4:1, 5:1, and 7:1 was 42%, 63%, and 82%; P digestibility increased as well. This was similar to mammalian hindgut fermenters, such as rabbits and horses, in which increased dietary Ca concentrations result in increased digestibility. Excessive Ca is excreted mainly through the urine. An optimal Ca/P ratio of 4:1 to 6:1 is recommended. Oversupplementation may result in Ca concretions in the bladder and could result in urolithiasis.

Frequent drinking of water was observed in Aldabran tortoises, and it is recommended that juvenile giant tortoises in general should always have access to water.⁴

BREEDING GIANT TORTOISES

Captive management of giant tortoises has a relatively long tradition in European zoos. In 1960 a census showed 30 Galápagos tortoises in 13 European collections, with the majority being males.¹⁵ The reason for this male dominance was that zoos were mostly interested in exceptionally large specimens. Because of the longevity of giant tortoises, this bias toward males still influences the gender ratio of the captive giant tortoise population. Management of these male-dominated groups was often unsatisfactory. In the summer the animals were kept outside and had barely heated shelters for colder days. In winter they were confined in small, overheated, humid shelters. Under these circumstances, it is surprising that the first successful breeding of Galápagos tortoises had already occurred in 1939 at North Miami Zoo and the Bermuda

Aquarium. Since then, breeding of Galápagos tortoises has been successful at the San Diego Zoo (1958), Honolulu Zoo (1967), Philadelphia Zoo (1975), Gladys Porter Zoo (1986), Life Fellowship Bird Sanctuary Florida (1987), and Zurich Zoo (1989). It is only since the 1980s that some institutions have had regular breeding success. Between 1990 and 2003, Zurich Zoo raised 50 Galápagos tortoises.

The first breeding of Seychelles tortoises outside their natural habitat was in 1976 in Sydney. Currently, regular breeding occurs only in Mauritius and Seychelles, with occasional reproduction in Britain, the United States, Australia, and Japan. Despite these highlights, reproductive success under captive conditions is still low compared with the number of giant tortoises kept in captivity.

Reports from the Seychelles Tortoise Conservation Project suggest that spatial and social variability plays an important role for successful reproduction. It is recommended that social groups should not be heavily male biased. One male should be significantly larger than the others to reduce aggressive encounters within the herd.¹⁰ There seems to be a hierarchy, with the largest male mating with the most females. Chida⁴ hypothesized that females with a carapace length more than 70 to 80 cm lose their breeding capability because they chose larger males, which at a certain carapace length would not be possible. Females must be able to avoid males, and male-male competition has been shown to stimulate breeding. Keeping large groups of at least 12 animals together appears to increase breeding activity. Best breeding was found where animals had much space (30 m² per animal).

Mating behavior may be observed during the whole year, but copulation does not always take place. Most mating is observed in the summer months when temperatures are above 23° C (73.5° F) in the morning and the late afternoon. In the Northern Hemisphere, mating season is from June to October. Oviposition takes place between November and March, usually during the night. In the Southern Hemisphere, copulation takes place from February to May, and oviposition is from June to September. Several days before egg laying, the female exhibits more active behavior, wandering around, sniffing, and testing the nesting area. In the absence of such an area, it has been observed that eggs are laid anywhere.

Ultrasound examination in Galápagos tortoises has shown that follicles became preovulatory at a diameter of 40 to 42 mm, and eggs were laid 34 to 84 days after thin-shelled eggs were detected in the oviduct.³ Eggs with shells may also be retained until the next breeding season, without adverse effects. Dystocia has

not been reported, but the risk is certainly increased if no adequate area for oviposition is offered.

For successful artificial incubation of eggs, Seychelles tortoises need at least 80% humidity and temperatures of 28° to 31° C (82.4°-87.8° F). Temperatures above 29° C (84.2° F) appear to result in females.¹⁰ At temperatures between 28° and 30° C, incubation lasts 125 to 136 days, whereas at temperatures between 30° and 32° C (86° and 89.6° F), hatching takes place after 90 to 94 days. At hatching, Aldabran tortoises weigh 40 to 70 g and have a carapace length of 50 to 80 mm.

At Zurich Zoo, Galápagos tortoise egg incubation is at 29° to 32° C with 65% humidity. Once daily the incubator is ventilated. Incubation lasts 105 to 164 days and has led to a majority of female offspring. Incubation and hatching takes place in a dark environment. At hatching, Galápagos tortoises weigh 50 to 80 g and have a carapace length of 60 to 80 mm.

Candling of eggs has been described, and it appears that within 3 to 4 weeks, fertile eggs may be recognized by the opacity of eggs compared with unfertilized eggs.

From pipping to hatching, it takes approximately 2 days. In our experience it is neither necessary nor advisable to help hatching animals. After hatching the tortoises are left in the incubator for up to 5 days, when the yolk sac has been completely absorbed. After 5 days the animals are offered food for the first time.

The young tortoises are kept in groups in boxes, with floor heating at 26° C. An IR and a full-spectrum lamp illuminate the enclosure. A 12-hour day/12-hour night photoperiod is chosen. The substrate is coarse gravel with a diameter of 5 mm, which is thought to reduce the risk of constipation and erosion by quartz sand, as seen in other land tortoise hatchlings. Other institutions have used alternate flooring material, such as grass hay, carpet, and rabbit pellets. Alfalfa pellets do not appear to be suitable as litter because of their high protein level, which may lead to developmental disorders resulting from fast growth. Young Galápagos tortoises are shy compared with Mediterranean tortoises (*Testudo* spp.) and often retreat under the vegetation or within holes. It is recommended to soak juvenile giant tortoises at least twice weekly in luke-warm water for an hour because this improves hydration and stimulates defecation. When the tortoises reach a straight carapace length of 35 cm, they have successfully been kept together with adult animals.

GROWTH OF GIANT TORTOISES

The major challenge in raising giant tortoises is their tendency for fast growth. At age 15 to 20 years, fast growth is slowed, which coincides with reaching



Fig 18-3 Measurement of straight carapace length in 1-year-old Galápagos tortoise (*Geochelone nigra*).

sexual maturity. Giant tortoises on Aldabra on a poor diet and little water grew to 20 to 23 cm of straight carapace length (sCPL) at age 4 years.

Under captive conditions, we determined that juvenile Galápagos tortoises at age 4 years were twice as long and weighed 10 times more compared with animals under natural conditions at the Charles Darwin Research Station on the Galápagos Islands.⁹ This phenotypic plasticity enables the tortoises to adapt their growth to their feeding conditions. A faster growth may lead to sexual maturity at younger age and shorter life expectancy, based on findings in gopher tortoises (*Gopherus polyphemus*) and crocodiles.⁹

Because fast growth may also result in developmental disease such as pyramiding, which cannot be reversed, it is advisable to monitor carefully the growth of juvenile giant tortoises. Up to age 4 years the tortoises are measured (Figure 18-3) and weighed at least twice a year at Zurich Zoo. The following formulas exist to assess the relation between body weight and carapace length (Jackson's ratio for Seychelles tortoises):

$$\text{sCPL} = 17.5 \times W^{0.345}$$

where sCPL = straight carapace length in cm and W = weight in kg,²⁴ and:

$$W = \text{cCPL}^3 \times 0.075$$

where cCPL = curved carapace length in cm and W = weight in grams.

Biting

When food amounts were reduced at Zurich Zoo with the aim to reduce growth rates in Galápagos tortoises, increased occurrence of biting between animals was observed. This has not been reported from other institutions that breed giant tortoises. Biting was usually



Fig 18-4 Wooden barriers in this enclosure of juvenile Galápagos tortoises (*Geochelone nigra*) help reduce the incidence of biting.

directed against the head or the limbs. Stress, resulting from boredom, hunger, or overcrowding, may predispose to biting. This type of aggression should not be confused with cannibalism, which has been observed in fast-growing lizards that appear to have a high caloric demand for development.⁶ The wounds are superficial but, if repeated, lead to scar formation. At Zurich Zoo the problem could successfully be controlled by introducing board “fences” in the cage, which allow the animals to hide themselves (Figure 18-4). In addition, large pieces of carrots ($6 \times 0.5 \times 0.5$ cm) were offered, which occupy the animals for a longer time.

RESTRAINT AND HANDLING

Mechanical Immobilization

Small giant tortoises may easily be immobilized, as done with the more common tortoise species. The animals are docile, and the risk of biting is minimal. Careful handling is important with tortoises up to age 2 years because the plastron is still soft and compression of inner organs may be a risk. Mechanical immobilization (e.g., for weighing or radiographs) of any size of tortoise may be achieved by positioning the animal on a flowerpot (Figure 18-5).

For dorsal positioning of larger animals, an automobile tire is practical. In addition, the juvenile animals should be trained to elevate the carapace from the ground when being scratched on the neck or when food is offered. This training is helpful when they become adults because it allows certain medical procedures (e.g., ultrasound examination of coelomic cavity) without stress.



Fig 18-5 Mechanical immobilization of adult Galápagos tortoise (*Geochelone nigra*).

Anesthesia

General anesthesia has been carried out in giant tortoises age 1 year and older, with a body weight of 300 g and larger. For endoscopic examination the author repeatedly and successfully used a combination of medetomidine (0.1 mg/kg body weight intramuscularly [IM]) and ketamine (10 mg/kg IM), which led to deep sedation, followed by intubation and isoflurane anesthesia. The medetomidine was antagonized with atipamezole (0.5 mg/kg subcutaneously). In larger animals, propofol (5 mg/kg intravenously) has also been used.

NONINFECTIOUS DISEASES IN JUVENILE GIANT TORTOISES

Giant tortoises are arguably the land-living vertebrate that shows the highest increase of body weight from birth to adult. With a weight at hatching of 80 g and an adult weight of 250 kg, this represents an increase of more than 3000 times! In comparison, an elephant, which is born at approximately 100 kg, increases its weight by only 40 times until adulthood.

From reports in rearing of captive giant tortoises, it appears that developmental diseases, especially *metabolic bone disease* (MBD), represent a major problem.

Developmental Metabolic Disease

Metabolic developmental disease has not been observed in the wild. MBD is not actually a single disease but a collection of medical disorders that affect the integrity of the skeleton. In tortoises, MBD also includes also the development of the carapace and plastron. Mader¹⁷ emphasizes that it is important to differentiate between MBD of *nutritional* (NMBD) and *renal* (RMBD) origin.

In growing giant tortoises, NMBD is predominant and presents as different stages, from hypomineralization to fibrous osteodystrophy of bones and pyramiding of the carapace. *Pyramiding* is characterized by excess growth of scutes on the carapace, with resultant pyramid shape of each scute.

No single etiologic factor was identified that causes developmental disease in giant tortoises. Also, no scientific experimental data are available regarding the prevention or treatment of developmental disease in giant tortoises.

A major predisposing factor is probably the phenotypic plasticity that enables giant tortoises to grow at an increased rate when food is available in large amounts. Under captive conditions this is usually the case, and furthermore, hatchlings do not need to be very active for feeding. It appears reasonable to reduce food intake to a minimum, which is achieved by the amount and the digestibility.

High protein levels should be avoided in the diet because of a repeated link to pyramiding and developmental disease. Hauser et al¹⁴ described four Aldabra tortoises imported at age 6 months that developed fatal MBD within 10 to 12 years.¹⁴ All animals showed softening of the carapace, pyramiding, and histologically diagnosed fibrous osteodystrophy. The diet consisted of roughage, produce, a mineral, and vitamin mix. Three times a week, 1 kg of minced meat and one to three eggs were offered to 15 animals. It appears reasonable to assume that these animals had excess protein in their diet. The role of protein level in the diet is controversial. Galápagos tortoises of the same age did not develop any disease, although they were kept under identical conditions. In African spurred tortoises (*Geochelone sulcata*), no effect of varying protein levels between 14% and 30% on a dry-matter basis were found over 5 months.²⁵ In the same study, lower levels of humidity resulted in significantly increased pyramidal growth of the carapace. Further studies are warranted to corroborate these findings in relation to giant tortoises. In the meantime, it is advisable to provide juvenile giant tortoise areas with at least 50% relative humidity.

Other factors have also been suspected to lead to developmental disease. Häfeli and Zwart¹² described in juvenile *Testudo* spp. that endoparasites, nephropathy, and typhlohepatitis are possible causes for pyramidal shell development.¹² In addition, young giant tortoises should receive adequate UV illumination. Because no experimental data are available on what is "adequate," exposure to direct sunlight should be made available whenever possible. At times this may be difficult, because when animals are kept under temperate con-

ditions and the use of UV-permeable roof systems may not result in an adequate photoenvironment. Analyses by Ebersbach⁷ at Hannover Zoo showed that approximately 30% of UVA and UVB light passed through such a roof. In the absence of experimental data, special UV light systems for reptiles must be installed at a suitable distance from the animals to offer adequate UVA and UVB light. At Zurich Zoo, high-intensity discharge mercury lamps are used in conjunction with IR heating lamps at a distance of 150 cm from the animals. The distance is critical because UVB burns may occur.

Institutions that breed giant tortoises do not keep juvenile animals in the same enclosure with adults. At Zurich Zoo, young Galápagos tortoises are kept for several years together with adults, until they reach a carapace length of 35 cm, which is within approximately 4 years. It is thought that this increases activity, which is considered beneficial for the skeletal development. No accidents have occurred.

Diagnosis of developmental diseases should be made as early as possible. Whereas pyramiding is readily visible, other forms of developmental disease may not be recognized until the disease has reached an advanced stage. Annual radiographic examinations with a dorsoventral beam to document skeletal development are part of the routine health examinations in juvenile Galápagos tortoises at Zurich Zoo (Figure 18-6).

If developmental disorders are diagnosed, therapy is challenging. A review of the feeding regimen and management is warranted, and corrections should be made where needed. Also, endoparasitic diseases should be ruled out. Enteral and parenteral doses of calcium and vitamin D₃ are warranted. Antibiotic therapy is indicated because secondary bacterial infections take advantage of the reduced immune status of the patient. If anorexia occurs, application of a permanent gastric tube, as described for other chelonians, may also prove beneficial in giant tortoises. Assisted feeding with a grass meal-based formula such as Critical Care (Oxbow Company, Murdock, Nebraska,) may be recommended. The total amount of 50 mL/kg is divided into four to six feedings.

INFECTIOUS DISEASES IN JUVENILE GIANT TORTOISES

Respiratory Tract Disease

The first two Galápagos tortoises that hatched at Zurich Zoo died at ages 14 and 15 months. The cause of death was a severe interstitial pneumonia. Stress

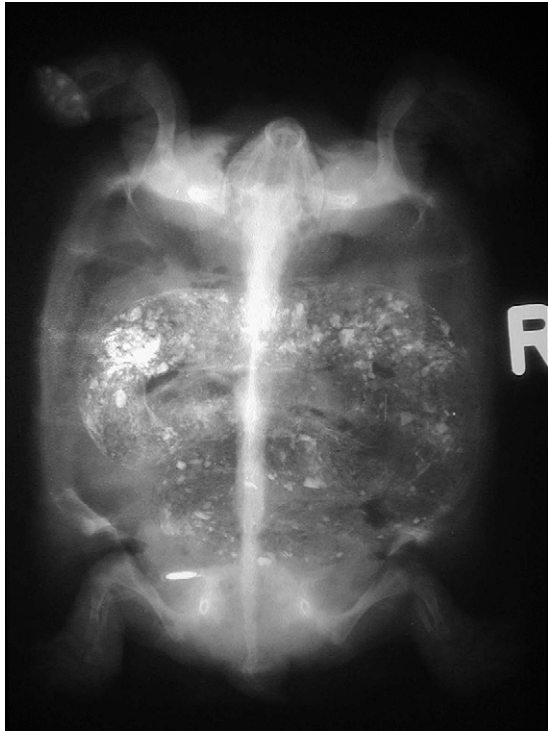


Fig 18-6 Dorsoventral radiograph of clinically healthy, 2-year-old Galápagos tortoise (*Geochelone nigra*). Note the large amounts of sand that are present in the large intestine. A microchip has been implanted in the left prefemoral area.

caused by increased growth rate may have been an important contributing factor by suppressing immunity in juvenile giant tortoises. Pneumonia is a well-recognized problem in general tortoise management.

Several factors contribute to respiratory tract disease. An important predisposing factor is the anatomy of the tortoise's lower respiratory tract. The trachea has only a primitive mucociliary apparatus, which acts poorly to clear inflammatory exudates from the lungs.²¹ Humid, cold weather was recognized as a causative agent for respiratory disease in giant tortoises at Phoenix Zoo. Other etiologies that may lead to pneumonia are endoparasites or ectoparasites and deficiencies in husbandry, sanitation, or nutrition.

A new form of stress may become important in the future as a result of growing transportation activities with juvenile giant tortoises. The increasing success in captive breeding results in larger numbers of juvenile tortoises being sent to other institutions. During quarantine, it is critical to keep animals in an optimal climatic and nutritional environment to minimize stress.

Although viral, fungal, and parasitic agents have been involved in respiratory tract disease, most cases of pneumonias in chelonians are caused by bacteria. Bacterial pneumonia may occur as a primary entity, as an extension of another disease process (e.g., infectious stomatitis), or as the establishment of a normal

commensal organism (e.g., *Escherichia coli*) in an abnormal location such as the lung.¹⁹

Clinical signs of pneumonia in chelonians usually appear late in the disease process. Inspiratory and expiratory dyspnea (extended neck), anorexia, and lethargy may be found. For diagnosis and follow-up of therapy, radiographic examinations are valuable; laterolateral and craniocaudal views will provide the most information. In addition, transtracheal or percutaneous lung washes are recommended for culture and sensitivity testing.

Antibiotic therapy follows the guidelines for other tortoises and the choice should be made on the basis of culture and sensitivity testing. Because pneumonia is usually advanced when clinical signs are apparent, initiating aggressive antibiotic treatment is generally recommended. Antibiotics may be given parenterally and by nebulization. Nebulization allows application at the site of infection.

Increased humidity also proves beneficial by promoting proper hydration of the respiratory epithelium and breakup of necrotic and inflammatory debris. Bactericidal agents are usually the drug of choice.

Enrofloxacin should be used cautiously. Casares and Enders² reported one case of an adult, 200-kg Galápagos tortoise that twice exhibited severe side effects after subcutaneous injection of 1000 mg and 500 mg of enrofloxacin (Baytril 5% and 10%). Signs started 1 hour after the injection and included hyperexcitation, uncoordinated movements, hypersalivation, and profuse diarrhea. At Zurich Zoo, oxytetracycline, 10 mg/kg IM every 48 hours, is usually the initial antibiotic drug of choice. The significantly increased body weight of giant tortoises compared with the tortoises usually kept as pets may necessitate allometric scaling of dosages to avoid overdoses. Supportive therapy should include daily soaking in lukewarm 0.9 % saline solution, subcutaneous fluids, and force feeding. Assisted feeding, as described earlier for MBD, may be necessary.

Endoparasites

Several institutions have reported endoparasitism in young giant tortoises. In the wild, tortoises have a balanced parasite burden. Under captive conditions, however, an imbalance is more likely to occur because of stress factors such as inadequate diet, low temperature, or overcrowding. An imbalance of endoparasite may represent a serious threat to giant tortoises and may predispose them to other diseases. *Entamoeba invadens* has repeatedly been diagnosed in juvenile Galápagos tortoises at Zurich Zoo without clinical

signs. Bloody diarrhea and subsequent dehydration are well-known signs. Premortem diagnosis is made by examining fecal samples. Samples should be fixed and shipped using sodium acetate–acetic acid–formalin (SAF) fixative. If *Cryptosporidium* spp. are to be found, a fresh fecal sample must be submitted. Treatment of *Entamoeba* was successful with metronidazole, 50 mg/kg orally (PO) for 10 days.

Coccidia are other protozoans that have been diagnosed in giant tortoises. Infestation without apparent disease is termed *coccidiasis*, in contrast to *coccidiosis*, which is used for clinical disease. In the absence of clinical signs, such as diarrhea or unthriftiness, treatment is not warranted.

Some parasites are important parts of the intestinal microflora, especially ciliates, such as *Balantidium* and *Nyctotherus*. Treatment should not be attempted.

Nematodes of the order oxyurids and strongylids have also been diagnosed in juvenile giant tortoises. The clinical significance is unknown. Fenbendazole (50 mg/kg PO for 5 days) and pyrantel (5 mg/kg PO with repeat treatment after 2 weeks) have been used to treat nematodes in juvenile Galápagos tortoises. Fenbendazole may need to be used cautiously in tortoises. A recent report revealed in Hermann's tortoises (*Testudo hermanni*) that were treated orally with two 5-day courses of fenbendazole 2 weeks apart at a dosage of 50 mg/kg an extended heteropenia with transient hypoglycemia, hyperuricemia, hyperphosphatemia, and equivocal hyperproteinemia/hyperglobulinemia, which were considered to be in response to fenbendazole administration.²⁰

Häfeli and Zwart¹² described in different *Testudo* spp. cryptosporidiosis and balantidiosis, which they considered etiologic agents besides nephropathies and typhlocolitis, for pyramidal shell development.

Therefore, it is safe to recommend routine fecal examinations and treatment of endoparasites if they are found. At Zurich Zoo, fecal examinations are performed twice a year.

CONCLUSION

Captive breeding and rearing of giant tortoises still represent a challenge, and little scientific evidence is available on the requirements of juvenile and adult giant tortoises. Zoo veterinarians must know that developmental diseases are the main risk in the rearing process. Respiratory tract disease, endoparasites, and biting are other pathologic conditions observed in juvenile giant tortoises. The fast growth of giant tor-

toises must be controlled by limiting the amount of food and reducing digestibility. Giant tortoises are well adapted to a high-fiber, herbivore diet. Special attention must be given to the mineral supplementation and photoenvironment.

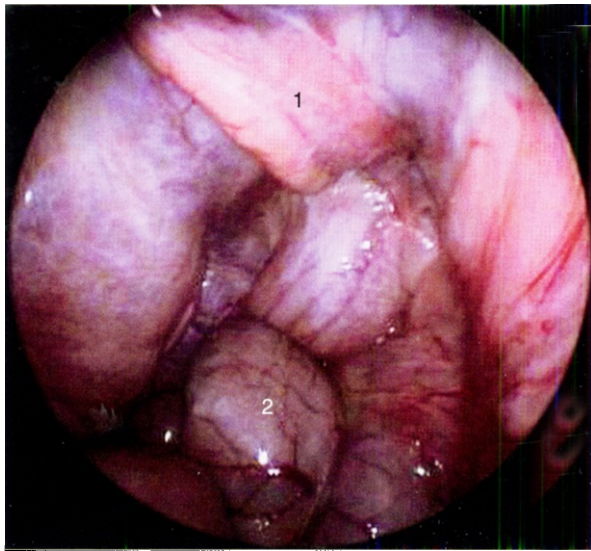
Acknowledgments

I thank Rene E. Honegger and Jürg Rohner for their valuable input.

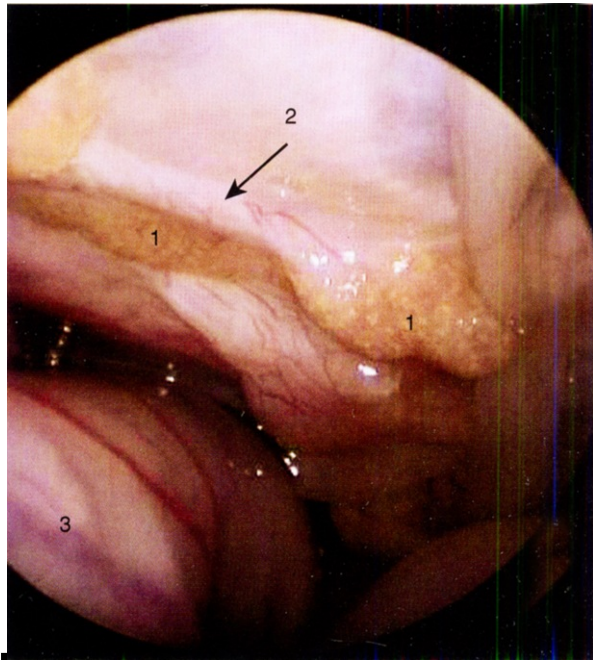
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Color Plate 18-1 Coelioscopy in 6-year-old Galápagos tortoise (*Geochelone nigra*). 1, Juvenile testicle; 2, intestine. (For text mention, see Chapter 18, p. 146.)



Color Plate 18-2 Coelioscopy in 4-year-old Galápagos tortoise (*Geochelone nigra*). 1, Juvenile ovary with primary follicles; 2, oviduct; 3, intestine. (For text mention, see Chapter 18, p. 146.)

CHAPTER 19

Reptile Protozoa

MARY C. DENVER

Many species of protozoa are found in reptiles. Some may be commensal or nonpathogenic organisms, and others are pathogens. The two most clinically important diseases are cryptosporidiosis and amebiasis. Each of these parasites may infect a wide range of reptilian species and are easily transmitted because of direct life cycles.

CRYPTOSPORIDIUM

Taxonomy and Life Cycle

Cryptosporidiosis is caused by parasites of the apicomplexan genus *Cryptosporidium*, which has a worldwide distribution. Thirteen species of *Cryptosporidium* are recognized, two of which, *C. serpentis* and *C. saurophilum*, are recognized as pathogens of reptiles.³¹ Nonreptilian *Cryptosporidium* spp. are not infective for reptiles.¹¹ Additional species likely exist that have not been completely described at this time. Infected snakes, lizards, and chelonians may become subclinical carriers or diseased.^{3,17,29}

The following life cycle is described for *Cryptosporidium parvum* in a mammalian host. The actual life cycle of *C. serpentis* has not been described. Infection occurs when the animal ingests a thick-walled, sporulated oocyst that excysts within the lumen of the stomach or intestine, producing four sporozoites, which penetrate the mucosal epithelial cells and develop into trophozoites inside the parasitophorous vacuoles. The trophozoites undergo asexual divisions to form merozoites. The merozoites invade nearby cells and either undergo asexual reproduction of more merozoites or form type II meronts, which in turn form the sexual stages, microgamonts and macrogamonts. After fertilization a zygote is formed and may develop into a thin-walled oocyst that is autoinfective or a thick-walled oocyst that is passed from the host. The cysts are infective immediately on passing from the host.⁷

Clinical Signs

Lizards and chelonians often exhibit poor growth or appetite, weight loss, or other, nonspecific findings, as well as postprandial regurgitation and passing of undigested food items.^{10,17,27} Snakes may be subclinically infected for years but capable of shedding the organism intermittently. Clinical signs of cryptosporidiosis in snakes may include anorexia, lethargy, intermittent or chronic regurgitation of undigested prey several days after feeding, chronic weight loss, and firm midbody swelling caused by gastric hyperplasia.³ Enteritis with severe inflammatory infiltrates without clinical or histologic signs of gastritis has been seen in wild-caught, rough green snakes (*Opheodrys aestivus*) and a garter snake (*Thamnophis sirtalis*). Sudden death was the presenting sign in these snakes.² Three iguanas (*Iguana iguana*) presented with aural-pharyngeal polyps.²⁸

Diagnosis

Diagnosis may be challenging because oocysts are shed intermittently.

Light Microscopy

Cryptosporidium spp. oocysts 4 to 8 μm long may be detected on light microscopy in unstained specimens of feces, mucus, or gastric lavage samples, although they may be confused with oocysts of other coccidia. Detection may be enhanced by the use of an acid-fast stain. Further increases in sensitivity may be achieved through the use of concentration techniques, such as Sheather's sugar flotation. The acid-fast stain and sugar flotation techniques have been published elsewhere.^{6,7} The use of a commercially available immunofluorescent antibody (IFA) stain (Merifluor, Meridian Bioscience, Cincinnati, OH 45244) increases the sensitivity of the test approximately 16-fold over acid-fast

staining for *C. serpentis*.¹⁴ The Merifluor IFA reacts weakly with the type of *Cryptosporidium* sp. typically found in geckos.¹² A positive finding on fecal or gastric wash samples does not necessarily indicate infection with *C. serpentis* or *C. saurophilum*. A negative finding may merely indicate lack of shedding.

In reptiles with mild subclinical infections, gastric lavage provides a better sample than feces or mucus. The lavage sample is best performed 3 days after feeding the snake. The increased gastric metabolism after a feeding is thought to increase *Cryptosporidium* metabolism, resulting in more parasites available for detection.¹⁶

Gastric Biopsy

It is not possible to determine whether cysts obtained from feces or gastric lavage are nonreptilian *Cryptosporidium* sp. cysts passing through the gastrointestinal (GI) tract, and therefore not infective to the host, or whether the cysts are *C. serpentis* or *C. saurophilum*, and thus represent an infection. Histopathology samples collected by gastric biopsy indicating that the gut epithelial cells are infected are necessary to prove the snake has cryptosporidiosis. Gastric biopsy may be performed using endoscopy or surgery.

Necropsy and Histopathology

The pathologic changes seen grossly have been covered extensively in the literature and include increase in the diameter of the stomach, edematous rugae, thickening of the gastric mucosa, decreased lumen size, mucosal petechiae, and focal necrosis in predominantly gastric cases and mild to severe enteritis in other cases.^{2,3} Histologically, in infected animals, the organisms are seen on the brush border of the epithelial cells. There may be little damage to the architecture in subclinical cases, or substantial changes may occur, including loss of the brush border, hyperplasia and hypertrophy of gastric glands, proliferation of gastric mucous cells, edema and inflammation of the submucosa and lamina propria, reduced luminal diameter, and inflammation of the mucosal layer.³

In reptiles with intestinal infection, few gastric lesions may be seen. A marked infiltrative enteritis is usually present, with abundant parasites in the enterocytes.^{2,17,27}

Treatment

Cryptosporidiosis is extremely difficult to eradicate. Anticoccidial drugs are not effective at eliminating

Cryptosporidium at nontoxic doses in humans or animals. Some pharmacologic agents may reduce the number of organisms or eliminate fecal shedding, but the organism is still present in the GI tract.^{6,15}

The most promising treatment for reptilian cryptosporidiosis to date has been the use of *hyperimmune bovine colostrum* (HBC); however, this product is not commercially available. HBC eliminated shedding but did not eliminate the organisms from the stomachs of snakes or the intestines of geckos within a 6-week treatment period. However, a longer treatment period may have succeeded in eliminating the organisms. HBC did eliminate infection in savanna monitors.^{12,13,18} Further work is needed in the area of treatment of cryptosporidiosis for all species.

Prevention and Management

The cysts are extremely hardy and resistant to standard disinfection procedures, including sodium hypochlorite (bleach) and povidone-iodine. Heat above 60°C, thorough desiccation, or freezing may be used for appropriate nonorganic materials. Ammonia and 10% formalin were effective after 18 hours of contact.⁴ At the Maryland Zoo in Baltimore, during the years of *Cryptosporidium* research, ammonia foot baths were used at the door of each reptile quarantine room; ammonia was used for disinfection of tools and equipment after removal of organic matter; separate tools were used for each room in quarantine; and the keepers were knowledgeable and well trained regarding prevention of transmission of *Cryptosporidium* organisms. Despite this, transmission occurred from one group of snakes to another snake housed in a different room.²

The safest way to eliminate cryptosporidiosis from a collection is through strict quarantine protocols with rigorous testing procedures and elimination of infected individuals. Reptiles with subclinical infections may appear healthy but serve as a source of infection for other reptiles for many years. Reptiles should not be euthanized based on the presence of *Cryptosporidium* in fecal or gastric samples without confirmation of actual disease in the stomach or intestine by biopsy and histopathology. It is difficult to justify the euthanasia of reptiles testing positive for cryptosporidiosis in quarantine unless the entire current reptile collection has previously been tested and found negative.

Endangered reptiles with cryptosporidiosis that are not considered candidates for euthanasia, even if infected, should be kept strictly isolated from noninfected reptiles. Periodic testing and treatment when

shedding may help prolong the quality of life and breeding potential of these endangered reptiles and may reduce transmission to the rest of the collection. There is no evidence of vertical transmission; eggs or offspring produced and removed from the adult environment immediately should have a reduced chance of infection.

ENTAMOEBA

Amebiasis may be a serious and devastating disease in a large reptile collection because of its ability to infect reptiles across taxonomic orders. Although morbidity is variable, the organism is difficult to eradicate from the environment or carrier animals once it enters a collection, and losses may continue for long periods of time. Snakes and lizards are considered most susceptible to fatal amebiasis. Although turtles, crocodilians, and reptile-eating snakes and lizards are reported to be resistant to the disease and potential carriers, it is also possible for them to develop clinical disease.³⁰

Taxonomy and Life Cycle

Cases of reptilian amebiasis with morbidity and mortality are usually attributed to the sarcodine organism, *Entamoeba invadens*, although there are multiple species of reptilian *Entamoeba* that not considered pathogenic in the normal host species.¹ The organism has a worldwide distribution. There is no reason to assume that *E. invadens* is the only pathogenic species of reptilian ameba, that all cases of clinical amebiasis in reptiles are caused by *E. invadens*, or that all strains of pathogenic ameba are equally pathogenic in all species of reptiles.

Entamoeba spp. have direct life cycles. The mode of transmission is fecal-oral contamination with quadrinucleate cysts. The reptile ingests a cyst that excysts within the lumen of the colon, releasing trophozoites. The trophozoites adhere to and lyse the colonic epithelium or aggregate in the mucin layer and reproduce, either invading adjacent mucosa and possibly migrating to other organs, or encysting and passing out of the host.²⁶ The organism does not reproduce well in snakes kept below 13° C or above 35° C.²³

Clinical Signs

Amebiasis causes morbidity and mortality by invading the epithelium of the colon, lysing the cells,

and attracting heterophils that contribute to damage to the colon. The extent of damage to the intestinal mucosa is variable but may result in thickening or obstruction of the GI tract or perforation. Secondary bacterial infection may be concurrent, worsening the clinical signs. Trophozoites may also enter the mesenteric circulation and invade the liver, peritoneum, and other sites, causing abscesses, thromboses, or other extraintestinal signs of amebiasis. Reptiles may show no clinical signs and may be found unexpectedly dead, or they may become lethargic, anorectic, and dehydrated. Mucus or blood may be seen in the feces.^{9,24,25}

Diagnosis

Antemortem Techniques

Detection of amebic cysts or trophozoites in fecal samples from clinically affected animals is considered diagnostic. Detection of carrier animals is difficult with currently available microbiologic techniques because of the low number of cysts usually shed. Trophozoites, which move by extending pseudopods, may only be detected in fresh fecal samples because they die rapidly once outside the host, usually within 20 to 30 minutes. The cysts are quite hardy and somewhat easier to detect. Staining with Lugol's iodine will make the cysts more visible by staining the nucleus; however, detection requires a high level of experience in technical staff.²⁶

Culture techniques may improve detection compared with direct and flotation fecal examinations but are extremely tedious and more useful for research than diagnosis. Results of the diagnostic evaluations performed at the Maryland Zoo in Baltimore indicate that 24° C is the best temperature at which to culture the organism, and that no diagnostic benefit is gained from culturing for more than 3 days because samples deteriorate over time after 3 days. The culture tubes should be centrifuged before removing samples for microscopic analysis to have the greatest chance of detecting cysts.⁵

In human medicine, antibody and antigen testing are the most useful methods for detecting amebic infection. Antibody testing using indirect hemagglutination (IHA), amebic gel diffusion test, complement fixation (CF), enzyme-linked immunosorbent assay (ELISA), counterimmunoelectrophoresis (CIE), indirect fluorescent assay (IFA), and latex agglutination (LA) could all be adapted for use in reptiles, but currently, no commercial reptile products are available. Antigen-based ELISAs are sensitive and specific and are easily

used by less experienced laboratory personnel.²⁶ Future work needs to be performed to determine whether any of these antigen-based test kits will work for *E. invadens* and whether they will be able to differentiate between *E. invadens* and nonpathogenic reptilian amebas.

Pathology and Histology

Gross postmortem findings may include thickening and edema of large intestine wall, erosions, ulcers, pseudomembrane formation, and liver abscesses. Histologic findings include fibrinonecrotic colitis, invasion of organisms into the submucosa of the gut, and in severe cases, enteritis, gastritis, hepatitis, and other areas of extraintestinal disease. Immunohistochemistry may be used to differentiate amebiasis from monocercomoniasis.²⁰

Treatment

Treatment recommendations for reptiles have been based on human treatment regimens. Metronidazole is the most important chemotherapeutic agent for invasive amebiasis. Recommended metronidazole dosages and frequencies for reptiles range from 275 mg/kg orally (PO) once⁹ to 20 mg/kg PO once daily for 5 days²¹ and many dosages and frequencies in between. Metronidazole is active against the trophozoites but does not eliminate cysts from the gut lumen. Metronidazole at high doses may cause neurologic signs, which were seen at the Maryland Zoo in Baltimore in adult black rat snakes treated at 250 mg/kg two times 2 weeks apart. Concurrent broad-spectrum antibacterial therapy is recommended in severe cases because of secondary bacterial invasion of the lesions.

In human clinical cases, metronidazole is usually given for 7 to 10 days and followed by lumen-active agents: paromomycin, diloxanide furoate, or iodoquinol (diiodohydroxyquine) to eliminate cyst passage in the feces. Paromomycin is the drug of choice in the United States for pathogenic human amebiasis. There are anecdotal reports of the use of these agents for the treatment of amebiasis in reptiles, although no clinical studies have been performed determining efficacy of these drugs for *E. invadens*.

The Maryland Zoo in Baltimore performed safety trials in juvenile elaphid snakes to evaluate metronidazole (50 mg/kg PO once daily for 5 days, repeated twice 2 weeks apart; 250 and 500 mg/kg PO every 2 weeks for three treatments), iodoquinol (30, 60, and 120 mg/kg PO once daily for 14 days), and diloxanide

furoate (20, 40, and 80 mg/kg PO once daily for 10 days). The higher doses of all drugs were evaluated for toxicity purposes. The lower doses were based on human pediatric drug doses. No snakes developed clinical or histologic signs of toxicity during the study. Efficacy was not evaluated because of the inability to culture a pathogenic strain to infect snakes during the study.⁸

In a separate study, 45 box turtles (*Terrapene carolina*) that were positive for *Entamoeba* sp. but did not have clinical signs were divided into three groups and treated orally once daily with metronidazole, diloxanide furoate, or iodoquinol at 50 mg/kg for 10 days, 40 mg/kg for 14 days, and 60 mg/kg for 10 days, respectively. None of the turtles developed any clinically observable side effects of medication, although some continued to shed organisms.⁸

Because it is unlikely that all carriers will be identified and cured, the goal of treatment should be to halt progression of clinical disease in affected animals and halt spread of disease to additional animals.

Two research projects have focused on treatment of human amebiasis, including development of a vaccine for *Entamoeba histolytica*¹⁹ and characterizing the organism's molecular biology to determine where new drugs may be able to interrupt the amebic life cycle. Some of this research is being performed using *E. invadens* as the experimental organism.²²

Prevention and Management

Quarantine procedures should include fecal parasitology; however, as previously stated, carriers are extremely difficult to detect. Separate tools should be used for each exhibit, or appropriate decontamination of tools should be practiced. Cysts may be transported between cages on arthropod vectors (roaches), indicating that pest control would also be helpful in controlling spread of disease. Mixed-species exhibits, although more aesthetically and educationally interesting, should be avoided. Drainage and filtration of water features in exhibits are also important.

One epizootic at the Maryland Zoo in Baltimore was attributed to an older water system in part of the reptile building. Six exhibits were connected by one drainage system. When one exhibit was drained, water would back up through the drains into the other exhibits on that side of the building. There were several mixed-species exhibits, including two containing crocodilian species and two with varanid species, connected by the drainage system. Multiple reptiles in these six exhibits developed clinical disease over several months.

CONCLUSION

Hygiene, sanitation, and excellent veterinary and husbandry practices are critical to reducing the risk of either *Cryptosporidium* sp. or *E. invadens* infection entering a collection. All new reptiles should be quarantined. Thorough quarantine screening of multiple fecal samples by experienced technicians will help reduce the likelihood of introducing infected animals to the collection. All sick reptiles should have multiple fecal examinations as part of the diagnostic workup. Animals diagnosed with either cryptosporidiosis or amebiasis should be isolated from the rest of the collection during treatment. Pest control is essential to reduce mechanical transportation of organisms. Best animal care practices would be to have separate tools for every enclosure and for staff to wash hands or change gloves between enclosures. Enclosures should be thoroughly disinfected and allowed to stand empty for several weeks to allow desiccation of any organisms that remain. Mixed-species exhibits combining chelonians and crocodilians with snakes and lizards should be avoided.

Additional research is needed to determine the most effective treatments for reptiles, including choice of pharmacologic agents, doses, and dosing intervals for treatment of both diseases. Further research is also needed to determine whether any of the currently available products for diagnosis of *Entamoeba histolytica* would be useful for antemortem diagnosis of *E. invadens*.

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Fluid Therapy in Reptiles

JUERGEN SCHUMACHER

Fluid imbalances are frequently seen in captive reptiles and are most often characterized by low circulatory fluid volumes. In many cases, suboptimal environmental conditions, such as low temperature and inadequate humidity, are major contributing factors to the development of anorexia, compromised immune status, and increased susceptibility to infectious agents. Most reptilian patients are presented in a state of chronic dehydration and volume depletion, often accompanied by severe electrolyte imbalances. In reptiles, trauma is a common cause of acute blood loss, whereas chronic blood loss is usually caused by conditions leading to decreased production or increased loss of red blood cells.

It is essential to identify the cause of dehydration and initiate corrective measures. Before treatment of other organ system abnormalities, a normovolemic state must be established. This state, along with optimal environmental conditions, will ensure optimal response to treatment, such as antimicrobial therapy and nutritional support. The general principles of fluid and transfusion therapy established in domestic animals also apply to reptiles.

Knowledge of species differences as well as the pathophysiology of reptilian diseases is essential for successful treatment of the reptilian patient presented with fluid imbalances or blood loss. Studies have shown that in reptiles the percentage of total body water (up to 75%) per unit of body weight is higher than in mammalian species. Compared with mammals, a higher portion of fluid is located within the intracellular space, although the interstitial fluid volume and plasma volume are lower in reptiles.⁸ Consequently, hypotonic fluids should be administered to reduce extracellular fluid osmolality and promote movement of water to the intracellular space. Hypertonic fluid solutions should be administered cautiously because they will cause fluid to move from the intracellular to the extracellular space.

THERAPEUTIC CONSIDERATIONS

The goal of effective fluid therapy is to restore normovolemia and maintain organ function, such as adequate perfusion of the kidneys. Fluid therapy should be initiated only when there is evidence of volume depletion. This mandates determination of the degree of dehydration and the electrolyte status of the patient. A thorough visual and physical examination will provide an estimate of the degree of dehydration. This information, combined with the history and the nature of the disease, may indicate the type of fluid and electrolyte imbalance.

Clinical signs of dehydration in reptiles include dry mucous membranes, eyes sunken into the orbit, decreased skin turgor, and a decrease in heart rate. Before initiating fluid therapy, it is mandatory to determine the degree of dehydration, and a venous blood sample should be submitted for hematologic and plasma biochemical determinations, including an electrolyte profile. These baseline values should be used to assess the degree of dehydration and monitor the success of fluid therapy. Although species differences are apparent, a packed cell volume (PCV) greater than 50%, increased plasma protein values (>6–8 g/dL), elevated levels of uric acid, and high creatinine values (>1 mg/dL) should be considered signs of dehydration.

BLOOD COLLECTION

Evaluation of a venous blood sample will provide the most accurate assessment of the health status of the reptile patient, including hydration status and organ function. As a general rule, a blood sample representing 1% of the total body weight of the reptile may be collected safely. Depending on the species, the total blood volume ranges between 5% and 8% of the reptile's body weight.

Collection of a venous blood sample for a complete blood count (CBC) and biochemical evaluations may be challenging in some reptiles because of their small size or the risk involved in restraining potentially dangerous species, such as venomous snakes or large crocodilians. In very small patients, it may only be possible to collect a single or a few drops of blood; however, this is sufficient to prepare a blood smear and fill a hematocrit tube for determination of the PCV and total protein.

In reptiles the anticoagulant of choice is lithium heparin because ethylenediaminetetraacetic acid (EDTA) has been shown to result in hemolysis of chelonian red blood cells. It is important to establish a protocol for handling and submission of reptilian blood samples for biochemical determinations. Blood should always be collected and handled using the same technique, including sampling site, collection tubes (plastic containers are preferred), and shipment to a laboratory.³ It is best to establish a protocol with the same laboratory, which will also assist in establishing a database for reference values.

In chelonians the preferred site for collection of a venous blood sample is the jugular vein. Additional sampling sites include the brachial plexus, the dorsal cervical sinuses (especially in aquatic turtles), the dorsal and ventral tail veins, and subcarapacial veins. Collection of a blood sample from the brachial plexus carries the risk of lymph contamination, which may alter hematologic and plasma biochemical parameters.

In snakes a blood sample may be obtained from the ventral tail vein or by cardiocentesis. With the latter technique, the snake is placed in dorsal recumbency, and the heart is located either by palpation or by visualization of the moving scales from the beating ventricle. The heart is then immobilized between two fingers, and an appropriately sized needle is inserted in the midline between two scales at a 45-degree angle, aiming cranially at the apex of the heart.

In lizards the ventral tail vein is the collection site of choice. The ventral abdominal vein is an alternate site for collection of a venous blood sample in most lizard species; however, the patient needs to be effectively immobilized to prevent excessive struggling. Crocodilians are typically bled from the supravertebral vessel located caudal to the occiput. The ventral tail vein may also be used for collection of a blood sample.

ROUTES OF FLUID THERAPY

In reptile patients, similar to domestic animals and avian species, the most effective, accurate, and pre-

dictable route of fluid therapy is through an intravenous (IV) or intraosseous (IO) catheter. However, catheterization of a peripheral vein may present challenges in reptiles because of their size and anatomy. For successful catheter placement, it is essential to be familiar with the normal anatomy and location of the vessel to be catheterized. Most approaches require a cutdown procedure, and local anesthesia should be applied with an effective agent such as lidocaine or bupivacaine. Care and maintenance of the catheter are similar to that for domestic small animal species.

In chelonians a venous catheter may be placed into the jugular vein and sutured or taped in place. The right jugular vein is better suited than the left vein and is usually readily visible underneath the skin. In lizards the large, ventral abdominal vein is my preferred site for placement of an IV catheter. With the lizard in dorsal recumbency, a 1- to 2-cm midline incision is made, and the vessel may be easily visualized. After placement of an appropriately sized catheter, it may be sutured, glued, or taped in place and connected to an extension set. In large lizards the cephalic vein may also be catheterized after a cutdown procedure. In snakes a cutdown procedure following aseptic preparation of the incision site is required to visualize and catheterize the right jugular vein. After catheterization the catheter should be sutured to the skin with the hub incorporated into the suture.

In lizards, crocodilians, and small chelonians an IO catheter may be placed as a useful alternative for IV access. The IO route of fluid and drug administration is almost equally effective as IV administration. After administration of a local anesthetic agent, an appropriately sized spinal needle is inserted into the tibia of lizards and crocodilians. In chelonians the tibia, femur, or the marrow of the shell may be used. Strict aseptic techniques should be followed to minimize complications such as development of osteomyelitis. IO catheters are most effective for constant-rate infusions because the bone marrow space is limited in reptiles and will not allow for large boluses of fluids. In green iguanas especially, care should be taken not to damage the bone excessively during catheter placement because many patients have metabolic bone disease.

Intracoelomic administration of fluids is more effective than subcutaneous (SC) or oral (PO) administration but less effective than the IV or IO route. In general, fluids are readily absorbed from the coelomic cavity; due to the ease of administration; however, care should be taken not to overload the animal with fluids. This is especially true for chelonians, and only calculated volumes of fluids should be administered. SC administration of fluids is recommended for maintenance

requirements and is not effective in cases of severe dehydration. PO fluid administration requires a functional gastrointestinal (GI) tract and is beneficial to meet maintenance requirements. In severely dehydrated patients, it may become necessary to administer fluids through several routes.

FLUID THERAPY FOR ELECTROLYTE IMBALANCES

Before fluid therapy, it is essential that the reptilian patient be warmed to the preferred optimal body temperature of the species. This ensures proper organ function as well as effective absorption and distribution of fluids. Fluid therapy should reestablish a normal circulating fluid volume, and for most reptilian patients, administration of a balanced electrolyte solution is indicated. However, a wide range of normal electrolyte values has been published in reptiles, and species differences are apparent.² Even for a specific species, interpretation of electrolyte values may be challenging because of seasonal, gender, and disease-specific differences.

For maintenance fluid requirements, it is recommended to administer 30 mL/kg/day, preferably as a constant-rate infusion.⁶ Selection of the type of fluid to administer depends on laboratory findings and the underlying disease process. Physiologic saline should be given for rapid expansion of the circulatory volume as well as for correction of hyponatremia and alkalemia. Lactated Ringer's (LR) solution supplemented with 2.5% dextrose will expand the circulatory volume without fluid shifts between compartments.^{5,7} LR solution alone expands the extracellular volume, but long-term administration may result in hypokalemia and therefore should be supplemented with potassium.⁶

Hyponatremia is most often caused by retention of water and edema. Reptiles with plasma sodium (Na^+) concentrations less than 130 mEq/L require therapy that should include diuretics and Na^+ -containing solutions. Patients with mild hyponatremia should be administered normal saline (0.9%), whereas severe hyponatremia requires administration of hypertonic saline. The following formula determines the amount of Na^+ to be administered in millimoles (mmol, mEq):

$$\text{Na}^+ \text{ (mmol)} = 0.2 \text{ BW (kg)} \times (\text{Normal } [\text{Na}^+] - \text{Patient's } [\text{Na}^+])$$

Reptiles are frequently diagnosed with *hypernatremia* ($\text{Na}^+ > 170$ mEq/L) because of chronic renal disease, dehydration, or GI disease. Hypernatremia results in

dehydration of the tissue caused by a shift of water from the intercellular space to the intravascular space. Clinical signs include seizures, coma, and depression. Effective therapy includes administration of a balanced electrolyte solution in hypovolemic patients, followed by treatment with 5% dextrose in water or 2.5% dextrose with LR solution. Care should be taken in chronically sick reptiles and therapy initiated gradually to prevent cerebral edema. In these patients, plasma Na^+ should be decreased at a rate of 0.5 to 1.0 mEq/L/hr.

In reptiles, serum potassium (K^+) concentrations less than 2.5 mEq/L and higher than 7 mEq/L require treatment. *Hypokalemia*, caused by GI loss of K^+ or low dietary K^+ levels will present as muscle weakness and intestinal ileus. Therapy for hypokalemia requires slow administration of potassium chloride (KCl) at a rate of 1 mEq/kg/day. *Hyperkalemia* is caused by renal disease or failure, extensive tissue trauma, or a ruptured urinary bladder. Clinically, anorexia, weakness, lethargy, and arrhythmias may be seen. Although normal saline is adequate to treat mild hyperkalemia, dextrose (to promote cellular uptake of potassium) or bicarbonate (HCO_3^-) is indicated in moderate to severe cases. In patients with severe arrhythmias, administration of calcium gluconate (10%) is indicated at a dose of 1.0 mL/kg.

In reptiles, *metabolic acidosis* associated with chronic renal disease and fluid deficits is often present. Whereas volume replacement therapy will address cases of hypovolemia, administration of sodium bicarbonate ($\text{base deficit} \times 0.3 \times \text{BW in kg} = \text{mEq NaHCO}_3^-$) will address hypervolemia. Clinically, *metabolic alkalosis* presents as weakness, lethargy, and in severe cases, seizures. For most reptiles, treatment includes expansion of the circulating fluid volume by administration of normal saline with KCl.

MONITORING

The success of fluid and electrolyte therapy may be difficult to assess, especially in small reptile species. Resolution of clinical signs of dehydration, such as decreased heart rate, dry mucous membranes, decreased skin turgor, and eyes sunken into the orbit, is the first indicator of therapeutic success. Repeat hematologic and plasma biochemical values, including electrolyte determinations, will accurately assess the patient's response to therapy. In smaller patients, in which the amount of blood that can be safely withdrawn is limited, essential electrolytes may be deter-

mined, or minimally, the patient's body weight should be accurately recorded.

TRANSFUSION MEDICINE

Information on transfusion of whole blood to reptiles is scant. Although no standard protocols have been published, whole-blood transfusions may be and should be performed in reptiles, especially in emergency situations. In practice, most reptiles are presented with a history of acute blood loss resulting from trauma or surgical procedures. The principles and techniques of transfusion medicine established in small animal medicine are also applicable to reptilian patients.

In reptiles and other species, complications are most often associated with improper collection, handling, and storage of blood before administration. Failure to follow aseptic techniques in handling blood may lead to sepsis. One-time transfusions minimize the risk of immunologic complications and have been successfully administered to a variety of reptilian species. Crossmatching is not routinely performed, but a slide agglutination test may be performed in patients that received prior transfusions.⁴ Complications may occur shortly after the start of transfusion, especially in patients sensitized by previous transfusions. Clinical signs include edema, hypotension, and intravascular or extravascular hemolysis.⁶ In these patients the transfusion should be discontinued and an immunosuppressive dose of a short-acting corticosteroid administered. In acute cases, immunologic or nonimmunologic responses may present as tremors, shock, and collapse of the patient and will appear shortly after start of the transfusion.

The patient should be evaluated before transfusion therapy, including identification of the underlying disease process as well as collection of a venous blood sample for hematologic and plasma biochemical determinations. The indication for transfusion of whole blood is determined by the patient's clinical status and a diagnosis of anemia, as indicated by a significant decrease of PCV. Before transfusion, at a minimum the PCV and total protein of the recipient should be determined. Normal values for both parameters have a wide range in reptiles (PCV, 20%-40%; total protein, 3-8 g/dL).² The type of anemia (e.g., hemolytic anemia, blood loss anemia) should also be determined before therapy.

In addition to laboratory findings, clinical signs supporting a whole-blood transfusion include tachypnea,

tachycardia, hypoxia, and hypovolemia. Reptiles presenting with acute anemia should receive a blood transfusion sooner than those with chronic anemia. Patients with acute anemia and a blood loss of more than 20% should be transfused with whole blood.⁶

Heparin is the most common anticoagulant used, and 5 to 10 U/mL blood is recommended. A citrate solution containing acid-citrate-dextrose (ACD) and blood at a ratio of 1:9 may also be used. Although no studies have investigated the viability of stored reptilian red blood cells (RBCs), blood collected from the donor animal should be used within hours of collection (not to exceed 6 hours) because the viability of RBCs decreases with the length of storage, and heparin, if used as an anticoagulant, does not contain preservatives. During storage the blood should be cooled and warmed before administration to the recipient.

The donor animal, of the same species, should be carefully selected and should be free of disease and in excellent health, as determined by physical examination, hematologic and plasma biochemical parameters, and fecal screen. The total blood volume in most reptiles accounts for about 8% of their body weight, and 10% of their calculated blood volume may be withdrawn safely.³

Whole blood should be administered through a large IV catheter to prevent hemolysis of RBCs and aggregation of platelets. For short-term venous access, a butterfly catheter may also be used. Alternately, blood may also be administered by an IO catheter. In snakes, venous access is difficult, and blood may be administered into the coelomic cavity. Before administration, blood should be slowly warmed to 25° to 30° C. Most blood transfusions in reptiles are administered by syringe, and the use of an inline filter (80 µm pore size) is recommended to prevent injection of blood clots and cellular debris.⁷ Reptiles presented in shock should receive a maximum of 20 mL/kg/hr of whole blood. In most patients, however, an infusion rate of 5 to 10 mL/kg/hr is recommended. In small reptiles especially, care should be taken not to administer excessive volumes of fluid because circulatory overload may lead to dyspnea, tachycardia, or death of the patient.

The success and efficiency of the blood transfusion should be monitored closely to avoid complications. A positive response to therapy, such as improvement of overall strength, pulse quality, mucous membrane color, and hematocrit, should be detectable within 2 hours after administration. If the patient shows no improvement with therapy, causes may include

continued blood loss, hemolysis, or decreased hematopoiesis, and further therapeutic and diagnostic measures should be performed.^{1,6}

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CHAPTER 21

Salmonellosis in Songbirds (Order Passeriformes)

JAMES K. KIRKWOOD

Salmonellosis has been recognized as a cause of disease and mortality in captive and free-living songbirds (order Passeriformes) for many years.^{5,13,17,19,20} *Salmonella typhimurium* is one of the species often found in birds, but a wide range of other *Salmonella* spp. have been isolated as well, some associated with disease and others from birds showing no signs. The tremendous growth in garden and backyard provisioning of wild birds worldwide has led to increased public observation and reporting of disease and mortality incidents in songbirds.^{1,7} Salmonellosis has been found to be one of the most common causes of these disease and mortality cases.^{3,4,5,12,15}

Approaches to the prevention and treatment of salmonellosis in captive songbirds have received considerable attention over the years.^{7,8,16} Recently, various efforts have been initiated to explore the epidemiology of the disease in free-living songbirds, some with a view to developing preventive measures when anthropogenic factors are implicated.^{7,12,14,15} However, the significance of the disease to the dynamics and viabilities of free-living populations has received little investigation. Outbreaks of salmonellosis may involve large numbers of birds and clearly have an impact on local population numbers at the time of their occurrence. At the individual level, it is reasonable to conclude from the nature of the clinical and pathologic findings that the disease has a severe impact on the welfare of affected birds.

ETIOLOGY

There are more than 2000 species of *Salmonella*. Some species, notably *S. typhimurium*, are frequently isolated from birds. Specific strains appear to be particularly pathogenic for various species of passerine birds. For example, mortality incidents in free-living greenfinches (*Carduelis chloris*) in the United Kingdom (U.K.) have been found to be commonly associated

with bacteriophage (phage) type 40. This strain has been diagnosed as the cause of mortality incidents in wild birds elsewhere in Europe and also in the United States and Canada. Other phage types isolated from songbird mortality incidents in Europe include 1, 41, 56 (variant), 121, 129, and 160.^{12,15} It is possible, and perhaps likely, that some strains that cause disease in some species of songbirds may be carried without causing signs in others. However, knowledge of which species of songbirds carry, and are susceptible to disease caused by various species and strains of *Salmonella* remains scant.

EPIDEMIOLOGY

Salmonellosis has been seen in a wide range of passerine species, and it is reasonable to expect that all are susceptible, although susceptibility may vary among species and with the strain of *Salmonella*. Among free-living garden birds, seed-eating passerines appear to be particularly at risk.^{6,12,14} In typical salmonellosis incidents in Northern Europe, most deaths occur in greenfinches, with chaffinches (*Fringilla coelops*), house sparrows (*Passer domesticus*), and other species being affected to a lesser extent. Where they occur, it appears that bullfinches (*Pyrrhula pyrrhula*), tree sparrows (*Passer montanus*), and siskins (*Carduelis spinus*) are also often affected.¹²

No structured surveys of the occurrence of outbreaks of salmonellosis in free-living wild birds have been undertaken, although one has recently been initiated in the U.K. (see www.ufaw.org.uk/gbhi.htm). However, this disease appears to be a common cause of epidemics in garden/backyard birds, especially during the colder months: December through to April in the Northern Hemisphere.¹² In one survey, *S. typhimurium* DT56 was isolated from 48%, 39%, and 13% of pooled feces samples collected from a bird table in 2001, 2002, and 2003, respectively, suggesting that that a potentially pathogenic strain is often carried and excreted.¹⁵

I found some evidence that infectious diseases (notably salmonellosis) caused a greater proportion of total mortality in gardens where songbirds were fed on a large scale (>1 kg of feed per day) than in those where less food was provided.¹⁰ This preliminary finding was consistent with a greater risk of infectious disease where large numbers of birds gather in relatively small areas to feed day after day. Whether outbreaks are typically caused by spread of infection from asymptomatic carriers or by exposure to strains from other sources (e.g., rodents, food) remains uncertain, but evidence suggests that some of the strains usually involved in garden/backyard bird mortality incidents (e.g., *S. typhimurium* phage types 40 and 56) are primarily associated with wild birds. Phage type 40 has also been isolated from game birds, horses, calves, and other domestic animals and can also cause disease in humans.

The main route of spread among birds is fecal-oral, and the potential for transmission clearly exists through fecal contamination at feeding and drinking sites. *Salmonella* spp. may survive and, in suitable conditions (e.g., in moist, uneaten food on a bird table on a warm day), multiply in the environment. The seasonal pattern of prevalence in free-living birds is probably attributable to the greater risk of spread of infection in the winter, when large numbers of birds gather at feeding sites, but other factors may also be involved in this seasonal pattern.

The incubation period (time from oral infection to onset of signs of disease) depends, among other factors, on the strain of *Salmonella*, the species and other features of bird infected, and the route and intensity of infection. The clinical signs can range from peracute to chronic, or infection may remain subclinical. The incubation period is typically 2 to 5 days in the acute disease.

CLINICAL SIGNS

The signs reported in both captive and free-living birds with salmonellosis typically include fluffed-up plumage, weakness, and lethargy.^{11,16} Other signs reported in songbirds with salmonellosis include difficulty in swallowing, polydipsia, anorexia, emaciation, profuse watery diarrhea, and pasty vents.² As with some other diseases, wild birds with salmonellosis are often observed to stay close to feeders or water baths and to continue to try to feed until just before death. In free-living populations, birds are often reported as being visibly ill for several days before they die, but some die more rapidly and while still in moderately

good body condition. In some cases, conjunctivitis, iridocyclitis, and panophthalmitis may occur.⁸

Observations in captive birds indicate that *Salmonella* spp. may cause disease ranging from mild enteritis to severe and generalized systemic disease.⁷ In some birds, chronic infections may occur with intermittent septicemia and clinical signs.⁸ In these cases, foci of infection may become established in the central nervous system (CNS) or joints, and granulomatous dermatitis also has been reported in some species.⁷

DIAGNOSIS

The pattern of the epidemic, clinical signs, and findings on gross postmortem examination may suggest salmonellosis. Isolation of *S. typhimurium* from fecal samples in clinical cases or from affected organs at postmortem helps to confirm the diagnosis. However, isolation of *Salmonella* does not necessarily confirm salmonellosis as the cause of disease because some birds are asymptomatic carriers. It has been found that fecal excretion may be intermittent, so several fecal samples may need to be examined before a provisional diagnosis of salmonellosis can be confirmed as more or less likely on the basis of fecal examinations.

Differential Diagnosis

The clinical signs are nonspecific. Differential diagnoses include other causes of generalized, and some localized, diseases. Where a number of birds are affected simultaneously, differential diagnoses include other infectious diseases and food-borne toxicoses. Other infectious diseases that may cause disease and mortality incidents with similar generalized clinical signs include colibacillosis and yersiniosis as well as some viral diseases, including avian influenza and Newcastle disease.

Necropsy Findings

At necropsy, affected birds are often found to be very thin, with loss of up to about one third of normal body weight. Many organ systems are affected, and typically the liver and spleen are enlarged, often with multiple, small, pale foci visible on external and cut surfaces. There may be air sacculitis, pericarditis, and ulcerations or granulomas of the esophagus and intestine. In some cases the CNS, joints, or eyes may be affected. In greenfinches the infection often causes

necrotic ingluvitis, with large, yellow swellings in the wall of the crop, and in some cases this appears to cause crop stasis; thus, although affected birds are usually very thin, their crops may be full.

THERAPY

Based on investigations of antibiotic sensitivity, some recommend long-term antibiotic treatment (e.g., 3-8 weeks) for the treatment of salmonellosis in captive wild birds.^{7,16} The disease is difficult to treat, however, and others believe that antibiotic therapy may be inappropriate. Gerlach⁸ justifies the use of antibiotics because this may reduce the risk of spread to human handlers and other species. However, studies have found that even after prolonged and apparently successful treatment, *Salmonella* may still be isolated from feces.¹⁶ Birds may be long-term carriers and shedders of the organism. During treatment and until repeated follow-up fecal sampling indicates that shedding has ceased, birds under treatment should be kept in strict isolation.

Treatment of free-living birds is not possible. In addition to the problems already mentioned, therapy depends on regular administration of antibiotics at safe but clinically effective doses and at correct dosing intervals, and this cannot be achieved by administration of antibiotics to free-living populations through food or water. Any such attempt would likely be counterproductive because it would be impossible to limit treatment only to affected birds, and it would likely promote the development of drug-resistant strains of the bacterium.

Efforts to ameliorate the welfare impact of salmonellosis in free-living wild birds are likely in most (if not all) cases to be better focused on preventive measures and the prompt euthanasia of birds that become severely ill and are unlikely to recover.

PREVENTION

Control and prevention of salmonellosis depend on minimizing the risk of transmission to susceptible birds. In the captive situation this involves strict biosecurity and hygiene measures. Captive populations should be kept separate from wild animals (vertebrate and invertebrate) that could introduce *Salmonella*, and birds should be permitted to join captive individuals or flocks only after quarantine and careful and repeated screening of fecal samples. Quality of food should be carefully controlled to minimize the risks of introduction of *Salmonella* by the food-borne route.

The spread of *Salmonella* and other infectious agents in fed wild birds is likely to be facilitated by crowding, particularly at feeding sites where foods can become heavily contaminated with droppings. When large populations of birds regularly feed at the same place, infected or carrier birds may transmit infection to susceptible individuals.^{9,10}

Although no studies have formally evaluated preventive measures for free-living wild birds, the following measures have been suggested^{7,11}:

- Where large numbers of birds visit garden/backyard feeding sites, provide food at several stations, and change these regularly so that they do not become heavily contaminated. Avoid feeding large numbers of birds on the same surface over long periods, unless it can be thoroughly cleaned.
- Suspended feeders should not have sills on which food particles and droppings may collect. The ground beneath these feeders, where birds often pick up morsels that have dropped, should be kept clean or the feeders moved regularly. Sweepings and brushings should be disposed of hygienically and ideally by incineration.
- Surfaces on which birds feed should be kept clean (e.g., by brushing them to avoid heavy fecal contamination). If placed on the ground, food should not be put in the same location day after day.
- Feeders and bird tables should be thoroughly cleaned on a regular basis (depending on how dirty they become) and cleaned daily if a disease outbreak occurs. After cleaning, feeders can be disinfected by washing or soaking for a few minutes in a 5% sodium hypochlorite (bleach) solution or using an alternative safe disinfectant. Feeders and tables should be rinsed thoroughly after disinfection.
- If water is provided in drinkers or birdbaths, it should be changed frequently, and these should be kept clean and regularly disinfected as previously described.
- Only fresh and good-quality food should be used.
- Food should be stored so that it cannot become contaminated by rodents or other pests.
- If signs of disease are observed, promptly try to diagnose the cause so that appropriate control and prevention measures can be implemented.

RISKS TO HUMANS AND OTHER ANIMALS

A wide range of species, including humans, are susceptible to some strains of *Salmonella* that may be carried by or may affect songbirds. Human cases of

disease caused by *S. typhimurium* phage type 40 are recognized by the U.K. Public Health Laboratory Service every year, thought to be associated with spread of infection from bird droppings. *S. typhimurium* has the potential to cause serious disease in humans, especially in young and older people, as well as persons with diseases that may compromise the immune system. Domestic dogs and cats may acquire the disease through eating carcasses of affected birds.¹⁸

Because of the zoonotic potential of some avian strains, it is important to advise members of the public, when involved in or instituting the previous measures to prevent salmonellosis in garden/backyard birds, to observe careful hygiene when cleaning bird tables, feeders, and water baths.¹

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CHAPTER 22

Veterinary Care of Bustards

THOMAS A. BAILEY

BIOLOGY

Bustards are small to very large terrestrial birds, chiefly inhabiting open plains and semidesert regions of the world, although some of the African species live in thick thorn scrub. Hunters have reported weights of 24 kg (53 lb) for male great bustards (*Otis tarda*) (Figure 22-1), which would place the bustard on a level with the mute swan (*Cygnus olor*) as the heaviest flying bird in the world.¹¹ Fossil records indicate that bustards originated in Africa, from where they diverged to occupy much of the Old World. No bustard species are found in the New World. There are 25 species of bustards,¹¹ but many subspecies are currently being reclassified as separate species, increasing this figure.

Bustards have long been associated with humans and have been represented in cave drawings, rock engravings, and church mosaics. The word *bustard* comes from the Latin *avis* ("bird") and a lost Spanish word, *tarda*, which is related to the English word "tread" and signifies "walking."¹¹ The earliest written accounts of bustards appear in the chronicles of medieval Arab and Western falconers. The Holy Roman Emperor Frederick II of Hohenstaufen, writing in the thirteenth century, was familiar with great bustards and was probably the earliest person to describe anatomic features peculiar to bustards in *De Arte Venandi cum Avibus*,³⁷ a medieval dissertation on falconry and hunting long recognized as the first zoologic treatise written in the spirit of modern science.

CAPTIVE BREEDING PROJECTS

Bustard populations are under pressure from agriculture, hunting, habitat modification, overgrazing, roads, building, and the spread of overhead cables.^{11,19} In recent years, there has been a surge of interest in the propagation of bustards in captivity, in particular, the houbara bustard (*Chlamydotis undulata*), the traditional quarry of Arab falconry, in the Middle East and North Africa. Programs for vulnerable and threatened

species of bustards such as the great bustard have been established in Europe and the former Soviet Union, and the kori bustard (*Ardeotis kori*) (Figure 22-2) in the United States (U.S.). Projects with threatened bustard species aim to produce surplus birds for release into protected areas, thereby supplementing declining wild populations, while houbara bustard projects in the Middle East and North Africa aim to provide surplus birds for sustainable hunting using falcons. Captive breeding programs in the U.S. for kori bustards and buff-crested bustards (*Eupodotis ruficrista*) aim to maintain populations that are genetically and demographically self-sustaining and do not rely on continued imports from the wild.¹⁷ Table 22-1 lists common and species names of bustard typically kept in captivity.

UNIQUE ANATOMY

A typical bustard has a short bill and a long, slender neck and is stout bodied, short tailed, and supported on long legs with only three toes. Unlike most bird species, bustards have hexagonal, not transverse-shaped, scales on their legs and no preen glands with which to oil their feathers. They compensate for their missing preen gland by producing powder down, and they maintain their feathers by grooming them with their bill, transferring the powder to all surfaces.¹¹

This section describes some unique anatomic features of the bustard alimentary tract. The alimentary tract of all species of bustards consists of a cavum oralis, pharynx, esophagus, proventriculus, ventriculus, small intestine (consisting of duodenum, jejunum, and ileum), large intestine (consisting of paired ceca and a rectum), and cloaca (Figure 22-3).

The cavum oralis of male kori and great bustards contains a throat sac, or saccus oralis. The opening to the saccus oralis is from a sublingual orifice between the rami of the mandible. It is used as a display chamber during the breeding season in these species. The saccus oralis is absent in many species, including houbara, white-bellied (*Eupodotis senegalensis*), and



Fig 22-1 Great bustard (*Otis tarda*) at the Great Bustard Trust, U.K. (See Color Plate 22-1.) (Courtesy Thomas A. Bailey.)

buff-crested bustards.⁶ Other species, such as the Australian bustard (*Ardeotis australis*), do not possess a true saccus oralis, and inflation of the throat area in this species during courtship is believed to be caused by distention of the esophagus.

Bustards do not possess a crop, and the esophagus connects the pharynx to the gastric region. The bustard proventriculus is small, whereas the ventriculus is comparatively voluminous, probably playing a role in food storage. In the absence of a crop and the presence of a well-differentiated ventriculus, drugs given orally pass rapidly through the esophagus and stomach and



Fig 22-2 Kori bustard (*Ardeotis kori*) in East Africa. (See Color Plate 22-2.) (Courtesy Paul Goriup.)

are available for absorption from the small intestine. Pharmacokinetic trials with orally administered enrofloxacin and amoxicillin have shown that these drugs are rapidly absorbed from the bustard gastrointestinal tract.¹

The ceca of bustards are well developed and indicate the dependence of the family on fibrous parts of plants.¹¹ The large intestine plays a major role in resorption of water. The rectum and cloaca form a greater proportion of the bustard alimentary tract compared with many other avian species, and this may reflect an adaptation for water conservation.

WEIGHT AND GENDER

Adult body weights of bustard species typically encountered in captivity are summarized in Table 22-1. Kori bustard chicks may be trained to stand on scales, reducing the need to handle birds to obtain an accurate weight.¹⁷

Adult bustards show sexual dimorphism. There is a seasonal variation in body weight and food intake in some species, including houbara and Australian bustards. Houbara bustards are heaviest in winter, progressively losing weight as summer approaches. Male Australian bustards have a 50% increase in body weight at the end of winter before they begin to display.¹⁴ Some smaller species (e.g., white-bellied bustards) may be sexed by differences in head and throat plumage. Larger species show strong, adult sexual dimorphism. Kori bustards are easily sexed at 1 year of age; although the plumage of both genders is similar, males are considerably larger than females. Juvenile bustards may also be sexed using endoscopy after about 6 months of age. Molecular sexing offers many advantages, being noninvasive, and if done from

Table 22-1**Average Adult Body Weight of Bustard Species Commonly Kept in Captivity**

Species (Scientific Name)	WEIGHT (kg)	
	Male	Female
Arabian bustard (<i>Ardeotis arabs</i>) ¹¹	5.7-10.0	4.5
Australian bustard (<i>Ardeotis australis</i>) ¹¹	5.6-8.2	2.8-3.2
Great bustard (<i>Otis tarda</i>) ¹¹	5.8-18.0	3.3-5.3
Great Indian bustard (<i>Ardeotis nigriceps</i>) ¹¹	8.0-14.5	3.5-6.8
Kori bustard (<i>Ardeotis kori struthinuculus</i>) [*]	10.0-18.0	6.0-7.0
Kori bustard (<i>Ardeotis kori kori</i>) [†]	7.0-14.0	3.0-6.0
Kori bustard (<i>Ardeotis kori kori</i>) [‡]	10.9-9.0	5.9
Heuglin's bustard (<i>Neotis heuglinii</i>) ¹¹	4.0-8.0	2.6-3.0
Nubian bustard (<i>Neotis nuba</i>) ¹¹	5.4	ND
Houbara bustard (<i>Chlamydotis macqueenii</i>) [§]	1.5-2.5	0.8-1.4
Bengal florican (<i>Houbaropsis bengalensis</i>) ¹¹	1.25-1.7	1.7-2.25
White-bellied bustard (<i>Eupodotis senegalensis</i>) [†]	0.94-1.4	0.91-1.16
Black bustard (<i>Afrotis afra</i>) [†]	0.7-0.8	ND
Buff-crested bustard (<i>Lophotis gindiana</i>) [†]	0.4-0.8	0.4-0.6
Little bustard (<i>Tetrax tetrax</i>) ¹¹	0.79-0.98	0.68-0.95

*Subspecies maintained in U.S. zoos.^{9,17}

†Subspecies maintained at National Avian Research Center (NARC).

‡Wild bustards.¹¹

§Asian houbara bustard (MacQueen's bustard) species.

ND, No data.

feathers or blood collected from freshly hatched chicks, it may allow different genders to be reared under different protocols.

SPECIAL HOUSING REQUIREMENTS

Differences exist in the housing of bustards both between and within species and according to the region where the birds are maintained in captivity.² Paillat and Gaucher²⁹ comprehensively reviewed the type and design of facilities for breeding houbara bustards in the Middle East. Great bustards in the United Kingdom (U.K.) have been kept as single-gender groups in 20 × 30-m grass paddocks topped with nylon netting and as a mixed-gender flock in a large, 4-hectare paddock.³ In the U.S., kori bustards are maintained in outdoor pens that are equipped with heated shed areas, where the birds are housed during periods of inclement winter weather. Bustards should be kept on well-drained ground. All species of bustard are susceptible to frostbite, and supplemental heat must be supplied when temperatures drop below 4°C (39°F). The provision of shade and shelter is important for birds managed outdoors in tropical and temperate

climates, respectively, as is the use of predator-proof fencing material. It should be remembered that in naturalistic aviaries, wild birds, including pigeons and doves, can be a potential source of disease for captive bustards (Figure 22-4)

NUTRITION

Diet of Free-Ranging Birds

Most bustards, including houbara bustards, are opportunistic omnivores, and in the wild their diet reflects the local and seasonal abundance of plants and small animals.^{19,36} Kori and black-bellied bustards (*Lissotis malanogaster*) will even eat carrion and have been found at the side of roads eating corpses of birds.¹¹ Plants appear to be a more important source of food for houbara bustards during winter and early spring, whereas animals, mostly invertebrates and small vertebrates, are more likely to be consumed in the late spring and summer.¹⁹ In the wild, houbara bustard chicks are fed mainly insects and small reptiles. Box 22-1 summarizes the diets in the wild of some species typically kept in captivity.

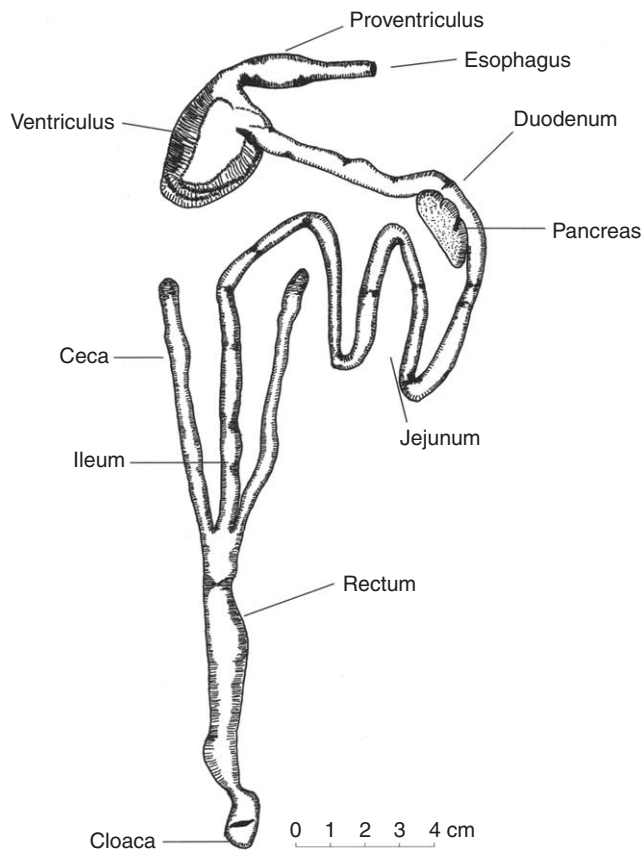


Fig 22-3 Alimentary tract of adult houbara bustard. (From Bailey TA, Samour JH, Naldo J, et al: *J Anat* 191:387-398, 1997.)



Fig 22-4 In these naturalistic aviaries, pigeons and doves may be potential sources of disease for captive bustards. Bustards managed in captivity are exposed to diseases that they would not normally encounter in their natural environment. (See Color Plate 22-4.)

Diet of Captive Birds

In captivity, bustards have been fed mice, mealworms, crickets, apple, cabbage, chopped greens, and bustard pellets, game bird pellets, or a mixture of crane and ratite pellets.³⁵ Captive bustards also eat invertebrates attracted to vegetation in naturalistic aviaries. Beef

Box 22-1

Summary of Wild Dietary Requirements of Some Bustard Species Kept in Captivity

Australian Bustard

A wide range of vegetable material and animals, including shoots, roots, leaves, flowerheads, seeds, berries, molluscs, myriapods, arachnids, insects (grasshoppers, beetles, caterpillars), reptiles, young birds, and rodents.

Buff-Crested Bustard

Diet is poorly studied, but it is known to eat seeds, green herbage, berries, *Acacia* gum, and insects (Tenebrionidae, scarabs, beetle larvae, grasshoppers).

Houbara Bustard

Diet of vegetable matter includes fruits, seeds, shoots, leaves, and flowers. Animals eaten include mainly Orthoptera, Coleoptera, and Tenebrionidae, as well as other invertebrates, small snakes, lizards, and hatchlings of ground-nesting birds (e.g., sandgrouse, larks).

Great Bustard

Mainly plant material and invertebrates, although small mammals, amphibians, and nestling birds are sometimes taken.

Great Indian Bustard

Mainly insects, including grasshoppers and beetles, but also scorpions, reptiles, bird eggs, and small mammals. Favored crop plants include groundnut, rocket salad, millet, and Bengal gram.

Kori Bustard

A wide range of vegetable material and animals, including seeds, berries, bulbs, *Acacia* gum, snails, insects, locusts, grasshoppers, dung beetles, rodents, lizards, snakes, and birds (eggs, nestlings, roadkills).

Little Bustard

Animals eaten include beetles, grasshoppers, and other terrestrial invertebrates. Plants eaten include shoots, clover, grain, leaves, flowers, and seeds.

Data from Collar NJ: Otididae (bustards). In Del Hoyo J, Elliott A, Sargatal J: *Handbook of birds of the world. Hoatzin to auks*, Barcelona, 1996, Lynx Edicions, pp 240-275; Johnsgard PA: Bustards, hemipods and sandgrouse. In *Birds of dry places*, New York, 1991, Oxford University Press, pp 106-115; and Tigar BJ, Osborne PE: *Ibis* 142:466-475, 2000.

mince has been used to replace mice or mealworms if either component is unavailable. Meat is a perishable food and, particularly in hot climates, needs to be handled carefully to prevent spoilage.¹⁷

Kori bustards kept in U.S. zoos are fed horsemeat in addition to mice, and the meat is supplemented with either crane and ratite pellets or game bird pellets.¹⁷

The mixture may be made into small meatballs and hand-tossed to each bird. This method of feeding facilitates close inspection of each bird. Kori bustards at the San Diego Zoo are fed on a mixture of a weight control diet for dogs (IAMS), Zoo Carnivore Diet 5 (Natural Balance), mealworm larvae, crickets, and whole adult mice (Edwards, personal communication). To encourage natural foraging behavior, supplement the daily diet and provide a form of enrichment; chopped green beans, hard-boiled egg, blueberries, giant grasshoppers, mincemeat, meatballs, lizards, feeder goldfish, cherry tomatoes, grapes, grains, shelled peanuts, sunflower seeds, and honeysuckle flowers are provided to kori bustards.¹⁷

In Europe, great bustards have been fed proprietary concentrate pellets, Zoo A diet (Mazuri Zoo Foods, U.K.), a proprietary crane diet, and a formulated bustard diet (Special Diet Services, U.K.), and fresh cabbage and cauliflower were fed ad libitum. Locusts, crickets, mice, dog food, insectivorous bird food, mealworms, spinach, cress, spring greens, and sprouting broccoli have been fed to increase the condition of the birds at the Zoological Society of London.³

In the Middle East at the National Avian Research Center (NARC) and the National Wildlife Research Commission (NWRC), maintenance pellets (14% protein) are fed to houbara bustards outside the breeding season, and productioner pellets (22% protein) are fed during the breeding season.^{29,35} Live food (mealworms or crickets) may be supplemented as part of taming protocols.

Small mammals are often fed to captive bustards. Pinkie mice are fed to smaller species of bustards, and fuzzy or adult mice are fed to the larger species such as kori bustards. Medications may be given to specific birds within favored items such as mice.

Hard, insoluble grit may be necessary for the grinding of fibrous feed in the ventriculus. Calcium carbonate may be added to the fresh food mixture to compensate for the calcium imbalance caused by the addition of live food or mincemeat. Also, in the U.S., crushed oyster shells are provided in kori bustard enclosures once a year as a calcium supplement.

RESTRAINT AND HANDLING

Correct methods must be used to catch and handle bustards to avoid injuries (Figures 22-5 and 22-6). The specific methods for catching birds depend on the species, age, level of tameness, aviary size, size of and environment within the cage/enclosure, and reason for the catch (translocation, medication, artificial



Fig 22-5 When restraining small to medium-sized bustards, the limbs can be tucked under the bird's body. (See Color Plate 22-5.)



Fig 22-6 A small cloth sack can be used to cover the head of medium-sized to large bustard species. It is important to have a hole so that the nares are not covered. (See Color Plate 22-6.) (Courtesy Thomas A. Bailey.)

insemination). Single, small or medium-sized birds in small aviaries may be captured by hand by one person if the bird is tame, or by using a net by one or more people if it is nervous. Nets may be used either with or without a handle. Larger species (e.g., kori bustards) housed in small enclosures may be caught by slowly guiding them into a small darkened shed, cornering them, and grabbing by hand around the body. Flocks of birds in large aviaries are often able to escape by flying or running, and they may be captured using

nets or “corrals,” which are blind-ended funnels, often made of shade cloth with a wide mouth and a circular catching area at the blind end.

Once captured, darkness has a quietening effect on bustards. This may be achieved by placing the bird in its capture crate into a dark room or by putting a hood over the head of the bird being handled. Falconry hoods are often used for medium-sized species, such as houbara and white-bellied bustards. Cotton bags with an opening over the nostrils to allow breathing may be placed over the head of larger species, such as kori bustards. Restraint harnesses are also useful for restraining hooded, small to medium-sized bustards. Degloving injuries of the skin over the legs may occur in birds that struggle and kick and that are not properly restrained. White-bellied bustards appear to be susceptible to this injury. Wrapping birds in towels is also helpful to reduce trauma and feather loss.

Chemical Restraint

Injectable Anesthetic Agents

Little has been published on accurate dose rates of injectable anesthetic agents in bustards (Table 22-2). When using injectable agents, an oxygen (O₂) supply and injectable doxapram should be available. Induction with midazolam and maintenance with isoflurane produces smooth anesthesia with good muscle relaxation, suitable for most surgical procedures. Alphaxalone-

alphadolone, a useful drug for short procedures, and the intravenous medetomidine-ketamine-midazolam combination are the injectable agents most familiar to me.

Gaseous Anesthetic Agents

The use of gaseous anesthetic agents in bustards allows more control of anesthesia than the use of injectable agents and is the preferred method for anesthetizing debilitated or sick birds. The combination of safety and rapid induction and recovery makes *isoflurane* the anesthetic agent of choice. Isoflurane allows a relatively easy method of induction by face mask for the small to medium-sized species of bustard. Once the bustard is induced, endotracheal intubation should be considered. Intubation of bustards is simple because of the large oropharyngeal cavity and the forward-placed glottis behind the base of the tongue. Intubation allows ventilation of the bustard in case of complications and also allows scavenging of waste gases. Generally, medium-sized bustards are induced at 5% isoflurane in 2 to 3 L/min O₂ and maintained at 2.5% to 3.0% in 1 to 2 L/min O₂. Buff-crested bustards usually need to be maintained on higher isoflurane levels of 3.5% to 4.0%.

It is also important to remember that during the breeding season, the vascularized saccus oralis of some larger species of bustard (e.g., kori) may occlude the glottis, leading to anoxia and death. Using an endotracheal tube in these birds is essential.

Table 22-2

Anesthetic Agents and Doses Used in Bustards

Drug (dose)	Route	Species	Comments
Alphaxalone-alphadolone (0.5-1.0 mL/kg or 4-12 mg/kg total steroid)	IV	HB, KB	Induction is rapid, and anesthesia lasts on average 10 minutes (range, 6-17 minutes). Suitable for short procedures such as endoscopy.
Midazolam (1-2 mg/kg) induction IV and isoflurane (1.5%-3.5%) maintenance	IV plus gas	KB, HB	Useful to induce smooth anesthesia; minimizes struggling. Midazolam at 2 mg/kg may result in prolonged recovery; thus using <1 mg/kg is recommended so that larger bustard species stand more quickly after recovery.
Medetomidine (65 µg/kg) plus ketamine (3.5 mg/kg) plus midazolam (0.5 mg/kg) Atipamezole reversal with a half-dose	IV	HB	Good combination for minor surgical procedures and endoscopy. Smooth anesthesia with good muscle relaxation. Disadvantage is need for IV administration set. May be maintained with isoflurane for longer procedures.

IV, Intravenous injection; KB, kori bustard; HB, houbara bustard.

COMMON SURGICAL TECHNIQUES

Captive bustards, particularly wild-derived birds, are frequently presented with keel and wingtip injuries, usually caused by trauma. Techniques for managing traumatic injuries in bustards are the same as those used in other species. Fractures of the carpometacarpus occur in juvenile bustards that are becoming more active and are experimenting with flight. This condition is often undiagnosed in bustards, and these fractures are best corrected by reduction and immobilization using an external splint or bandage.²¹

The ingestion of foreign bodies is a common cause of morbidity in bustards maintained in outdoor aviaries⁷ (Figure 22-7). Kori bustards often swallow sticks, nails, and pieces of wire, which may cause obstruction or may penetrate the wall of the proventriculus or gizzard and cause pleuritis or peritonitis. Lloyd²⁰ described the removal of a foreign body from the ventriculus of a black bustard using an endoscope

and grasping forceps. In medium-sized or large bustards, however, the length of the esophagus (>0.25 m in houbara bustards and >0.6 m in kori bustards) may preclude the retrieval of ventricular foreign bodies using rigid endoscopy, and ventriculotomy is the most likely technique to be used.

Surgical techniques to remove proventricular and ventricular foreign bodies have been described for cranes and ratites, and although the procedure is similar, anatomic differences should be considered when ventriculotomy is performed in bustards. In bustards the *tenuis craniodorsalis* and *tenuis caudoventralis* muscles are comparatively thin and are present in the saccus cranialis and saccus caudalis, which make up the tapering ends of the ventriculus (Figure 22-8).⁶ Bailey et al.⁷ reported the clinical findings in two kori bustards with ventricular foreign bodies and the successful surgical approach for removal of “hardware” from one bird.

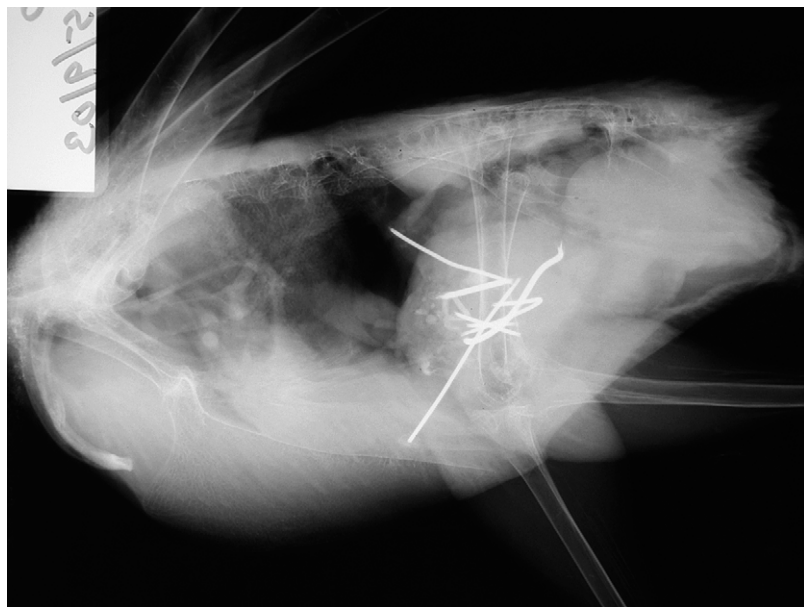


Fig 22-7 Radiograph of houbara bustard, lateral projection, with radiopaque particles in the ventriculus. This bird presented with weight loss, leukocytosis, and anorexia. It had been put into a renovated aviary that was subsequently found not to have been adequately cleaned after construction. In this case the wire had perforated the ventriculus, and the bird required surgery. (Courtesy Tom Bailey.)

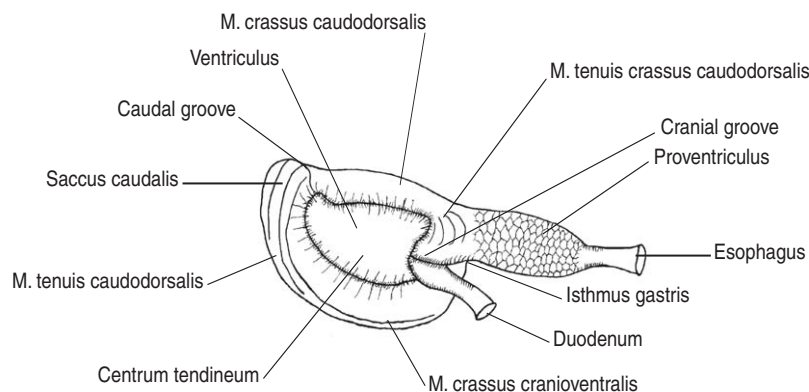


Fig 22-8 Stomach of houbara bustard (left lateral view). (From Bailey TA, Samour JH, Naldo J, et al: *J Anat* 191:387-398, 1997.)

DIAGNOSTIC TECHNIQUES

Bustard blood samples are usually obtained from the brachial vein or the medial metatarsal vein. Samples can also be collected from the right jugular vein in juvenile bustards. Tables 22-3 and 22-4 provide hematology and plasma biochemistry values, respectively, for selected bustard species. Bone marrow collection may be done as in other avian species. I favor aspirating marrow from the tibiotarsus with a sterile needle and syringe. Cytologic examination of wet preparations of oropharyngeal swabs is important in screening bustards for motile protozoal parasites. Fresh feces or a saline-moistened cloacal swab is examined for parasites and their eggs. Fresh feces are collected from the floor or ground of the enclosure or

aviary. Fecal samples should be assessed using wet preparations (motile protozoa), flotation techniques (parasite ova), and Gram or Rapi-Diff staining to quantify the presence of inflammatory cells, fungi, and bacteria.

DISEASES

Although bustards do not have many unique infectious disease conditions, a wide variety of diseases have been reported in both wild and captive species.² The diseases of houbara bustards have been best described because of the proliferation of well-funded breeding projects in the Middle East over the last 15 years.

Table 22-3

Reference Ranges* for Hematology Parameters in Selected Species of Adult Bustards

Parameter	Kori Bustard ¹⁸	Houbara Bustard ³¹	White-Bellied Bustard ¹³	Buff-Crested Bustard ¹²
Red blood cells ($\times 10^{12}/L$)	2.30 \pm 0.06 (1.74–2.95)	2.53 \pm 0.09 (1.95–3.15)	2.31 \pm 0.08 (2.16–2.47)	2.89 \pm 0.19 (2.00–4.27)
Hemoglobin (g/dL)	14.10 \pm 0.16 (11.9–15.9)	14.72 \pm 0.14 (13.7–15.7)	15.23 \pm 0.17 (14.9–15.5)	17.62 \pm 0.52 (15.50–22.20)
Hematocrit (L/L)	0.47 \pm 0.05 (0.395–0.525)	0.47 \pm 0.08 (0.42–0.51)	0.47 \pm 0.01 (0.45–0.50)	0.47 \pm 0.01 (0.40–0.50)
Mean cell volume (fL)	208.5 \pm 5.1 (161.9–275.4)	189.7 \pm 8.85 (146.3–259.1)	205.7 \pm 8.3 (190.2–218.6)	172.85 \pm 9.36 (105.39–220.00)
Mean cell hemoglobin (pg)	62.4 \pm 1.6 (48.0–84.6)	58.9 \pm 2.44 (46.6–74.3)	66.03 \pm 3.06 (60.3–70.8)	65.21 \pm 4.46 (40.71–97.50)
Mean cell hemoglobin concentration (g/dL)	30.0 \pm 0.4 (29.7–34.9)	31.16 \pm 0.54 (26.1–34.1)	32.1 \pm 1.0 (30.6–34.0)	37.63 \pm 1.18 (31.31–47.56)
Thrombocytes ($\times 10^9/L$)	5.5 \pm 0.7 (1.49–18.0)	6.82 \pm 0.59 (2.76–9.88)	5.99 \pm 2.9 (3.06–11.8)	8.81 \pm 1.04 (4.00–15.00)
Fibrinogen (g/L)	2.42 \pm 0.10 (1.42–4.5)	1.87 \pm 0.26 (0.8–4.8)	2.0 \pm 0.15 (1.7–2.19)	1.7 \pm 0.23 (0.66–4.31)
White blood cells ($\times 10^9/L$)	7.29 \pm 0.42 (3.05–12.85)	5.81 \pm 0.29 (4.25–7.6)	6.26 \pm 0.7 (5.2–7.6)	5.66 \pm 0.38 (4.00–9.80)
Heterophils ($\times 10^9/L$)	3.98 \pm 0.32 (0.95–9.25)	3.64 \pm 0.24 (1.99–4.82)	2.73 \pm 0.75 (1.92–4.25)	3.32 \pm 0.32 (1.44–5.88)
Lymphocytes ($\times 10^9/L$)	2.21 \pm 0.24 (0.41–5.45)	1.84 \pm 0.15 (0.97–3.24)	2.51 \pm 0.17 (2.18–2.73)	1.11 \pm 0.20 (0.31–3.03)
Monocytes ($\times 10^9/L$)	0.60 \pm 0.07 (0.0–1.57)	0.15 \pm 0.03 (0.0–0.42)	0.45 \pm 0.14 (0.22–0.72)	0.42 \pm 0.10 (0.04–1.30)
Eosinophils ($\times 10^9/L$)	0.35 \pm 0.05 (0.0–1.15)	0.07 \pm 0.01 (0.0–0.23)	0.24 \pm 0.09 (0.06–0.36)	0.24 \pm 0.04 (0.00–0.62)
Basophils ($\times 10^9/L$)	0.20 \pm 0.03 (0.0–0.80)	0.07 \pm 0.02 (0.0–0.26)	0.31 \pm 0.13 (0.07–0.54)	0.44 \pm 0.08 (0.10–1.23)

*Mean value \pm standard error of the mean (minimum to maximum values).

Table 22-4**Reference Ranges* for Biochemical Parameters in Selected Species of Adult Bustards**

Assay	Kori Bustard ⁵	Houbara Bustard ⁵	White-Bellied Bustard ²	Buff-Crested Bustard ³
Glucose (mmol/L)	14.16 ±0.33 (11.1–17.65)	16.89 ±0.26 (14.04–19.59)	19.09 ±1.05 (16.32–23.04)	20.36 ±0.55 (15.28–28.83)
Uric acid (μmol/L)	534.25 ±38.36 (237.92–969.52)	432.42 ±39.79 (202.23–1005.21)	408.63 ±15.39 (356.88–481.79)	533.93 ±38.97 (202.38–928.57)
Total protein (g/L)	30.0 ±0.82 (23.0–40.0)	37.93 ±0.9 (30.0–48.0)	30.0 ±1.71 (25.0–35.0)	32.81 ±0.83 (25.0–41.0)
Albumin (g/L)	11.0 ±0.3 (8.0–15.0)	14.5 ±0.28 (11.0–18.0)	12.0 ±0.9 (9.0–15.0)	12.91 ±0.37 (10.0–16.0)
Globulin (g/L)	1.9 ±0.06 (1.5–3.1)	2.38 ±0.09 (1.7–3.7)	18.0 ±0.89 (15.0–20.0)	19.61 ±0.64 (14.0–26.0)
Albumin/globulin ratio	0.58 ±0.01 (0.29–0.73)	0.64 ±0.03 (0.32–0.84)	0.66 ±0.03 (0.56–0.75)	0.67 ±0.02 (0.52–0.79)
Alkaline phosphatase (U/L)	65.9 ±2.6 (37–98)	80.39 ±7.24 (17–175)	44.17 ±10.53 (23–86)	475.16 ±45.71 (160–868)
Alanine transaminase (U/L)	34.4 ±3.43 (20–120)	45.14 ±3.27 (22–97)	ND	ND
Aspartate transaminase (U/L)	207 ±7.1 (168–369)	467.9 ±24.93 (246–774)	444.67 ±42.34 (266–574)	334.96 ±11.91 (217–482)
Lactate dehydrogenase (U/L)	818.2 ±50.8 (543–1921)	609.57 ±43.42 (246–774)	755.17 ±80.18 (474–985)	373.96 ±29.72 (152–788)
Creatine kinase (U/L)	275 ±87.07 (35–2527)	778.4 ±122.2 (12–2309)	377.14 ±42.77 (170–521)	361.65 ±41.27 (115–797)
Magnesium (mmol/L)	1.05 ±0.02 (0.85–1.27)	1.01 ±0.03 (0.81–1.27)	1.01 ±0.07 (0.84–1.32)	1.05 ±0.03 (0.83–1.36)
Calcium (mmol/L)	2.34 ±0.07 (1.71–3.44)	2.49 ±0.08 (1.46–3.01)	2.42 ±0.05 (2.25–2.56)	2.55 ±0.06 (2.02–3.51)
Cholesterol (mmol/L)	3.7 ±0.13 (2.4–5.15)	6.78 ±0.33 (4.06–10.65)	3.30 ±0.35 (2.66–4.97)	3.64 ±0.15 (2.19–5.37)

*Mean value ±standard error of the mean (minimum to maximum values).
 ND, Not determined.

Viral Diseases

Significant viral diseases of bustards include Newcastle disease (Figure 22-9), avipox, and avian influenza (Table 22-5). Reovirus has been implicated in losses in young Heuglin's bustards (*Neotis heuglinii*) at a facility in Dubai, United Arab Emirates, and since the initiation of a vaccination program using inactivated reovirus vaccine (Nobilis Reo, Intervet), no further mortality has been reported (McKinney, personal communication). Other viruses isolated from bustards include adenovirus, Gumboro disease virus, herpesvirus, and paramyxovirus type 2 (PMV-2), but the significance of these findings is as yet unknown.²⁷ Lymphoid leukosis has been reported in bustards. Serologic surveys of houbara bustards in the Middle East have shown of antibodies against Gumboro disease, pigeon herpes,

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Fig 22-9 Houbara bustard with Newcastle disease from paramyxovirus type 1 (PMV-1) infection, showing typical central nervous system signs of torticollis. (See Color Plate 22-9.) (From J Zoo Wildl Med 28(3):325-330, 1997.)

Table 22-5

Common Viral Diseases Reported in Bustards

Etiology	Epizootiology	Signs	Diagnosis	Management
Avian Influenza Orthomyxoviridae. Influenza A virus has hundreds of subtypes, with frequent appearance of new serotypes to which a population does not have immunity.	Reported in adult houbara and juvenile white-bellied bustards in the Middle East.	Morbidity and mortality vary with age of the bird and strain of the virus. Clinical signs consistent with mild to severe respiratory disease. Sudden death reported.	Virus isolation from cloacal or tracheal swabs. Virus may be isolated postmortem from trachea, lung, air sac, sinuses, spleen, liver, intestines, and cloaca.	Treatment is supportive. Infections in captive birds may be prevented by reducing exposure to free-living birds. Inactivated H9 and H5 vaccine available for domestic poultry have been used in bustards. Oseltamivir phosphate (Tamiflu, Roche), 10 mg/kg orally, has been used in the United Arab Emirates.
Newcastle disease Paramyxovirus (PMV). There are nine serotypes, but only types 1 and 2 have been isolated from bustards. The disease produced varies with the virus pathotype and other factors, including age, immune status, and general health conditions.	Transmitted primarily by respiratory aerosols, fecal contamination of food and water, direct contact with infected birds, and fomites. Common in the Middle East. Little is known about the incubation period in bustards, but in one outbreak, cases were diagnosed 4-17 days after arrival of birds at a quarantine facility.	Signs include ataxia, circling, depression, diarrhea, head shaking, head tilt, head tremor, inappetence, incoordination, nasal/ocular discharge, opisthotonos, recumbency, torticollis, head tucked under keel, and walking backward.	Diagnosis requires virus isolation and demonstration of the viral antigen by immunohistochemistry or rising antibody titers. Virus may be isolated from swabs of the feces and oropharynx in live birds. Samples from postmortem cases include brain, lung, liver, intestine, and spleen. Gross lesions at postmortem may be absent but may include pancreatic inflammation.	Control measures to exclude PMV-1 from collections include strict quarantine protocols for incoming stock, exclusion of free-living birds, and vaccination, using commercially available inactivated poultry vaccines (1.0 mL/kg Newcavac Nobilis, Intervet). In other avian species, birds that have survived infection with PMV-1 may become carriers and intermittently shed virus. In a study on houbara that recovered from PMV-1 infection, virus was not isolated from surviving birds for 11 months after initial infection.

Continued

Table 22-5—cont'd

Common Viral Diseases Reported in Bustards

Etiology	Epizootiology	Signs	Diagnosis	Management
Avipox				
Avipox virus is a large DNA virus that induces intracytoplasmic inclusion bodies called Bollinger bodies.	Transmission occurs through latently affected birds and biting arthropods (e.g., mosquitoes). Direct transmission of the virus is also possible and is linked to traumatic injuries. Although bustards of any age may be affected, juveniles and debilitated birds are particularly at risk. Avipox virus may remain latent for years, and nonspecific factors are associated with viral reactivation.	Four forms of disease occur in bustards: <ol style="list-style-type: none"> 1. Cutaneous or drypox. Common form characterized by papular lesions on the nonfeathered parts of the skin. 2. Diphtheroid or wetpox. Characterized by inflammation of the conjunctiva, eyelids, buccal cavity, larynx, trachea, bronchi, and esophagus with fibrinous plaques. 3. Septicemic form. Characterized by acute-onset lethargy, dyspnea, anorexia, cyanosis, and death. Birds may die acutely without clinical signs. 4. Tumors. Large, tumorlike nodular lesions occasionally observed. 	Diagnosis may be made through histopathologic examination of biopsy samples and demonstration of typical cytoplasmic inclusions or Bollinger bodies. Virus isolation may be performed using biopsies from wet or dry lesions, or from visceral organs in the septicemic form. Virus may be isolated from cloacal swabs of birds with the wet and septicemic forms of avian pox. Electron microscopy is important because in some cases, virus cannot be isolated from dry scabs.	Bustard chicks may be vaccinated from about 10-14 days of age in high-risk environments. No side effects have been seen in bustards using pigeonpox, fowlpox, and canarypox (Kanapox, Merial) vaccines, but the efficacy of such vaccines is currently unknown. The majority of large houbara breeding flocks use canarypox vaccine. ²⁸ Mosquito control should include netting around rearing houses. Sparrows have been implicated as the source of virus at one project. Control of the sparrows contributed to a decreased incidence of pox at this center.

infectious bursal disease type 2, and Marek's disease viruses.^{2,27}

Recently, I vaccinated bustards in Dubai against avian influenza using commercially available poultry vaccines. Losses in bustards and other avian species at an avicultural facility were associated with a low-pathogenic, H9N2 strain of avian influenza. Bustards were vaccinated against the H9N2 strain with 0.5 mL of Gallimune 208 ND plus Flu H9 M.E., given subcutaneously (Merial, Lyon, France). These birds were also vaccinated against the H5N2 strain of avian influenza with 0.5 mL of Nobilis Influenza H5 (Intervet, Boxmeer, Netherlands), also given subcutaneously, as a precaution against the regional threat currently from this disease. Birds were given a second booster vaccination 4 weeks after the first vaccination. No side effects

were seen in vaccinated birds. Obon et al. confirmed serologic conversion in houbara and white-bellied bustards after vaccination with this H5N2 vaccine.²⁵ Seroconversion has also been reported in a variety of nondomestic avian species given commercially available H5N2 and H7N7 poultry vaccines.^{26,30}

Bacterial Diseases

Studies on captive and free-living bustards have shown that healthy bustards are colonized by a wide range of bacteria. *Proteus* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Aerococcus* spp., and *Enterococcus* spp. should be considered part of the normal aerobic intestinal flora of captive bustards.³⁴

Box 22-2

Features of Chlamydophilosis in Bustards

Chlamydophila psittaci (formerly *Chlamydia psittaci*) is an obligate intracellular bacterial parasite often associated with morbidity in smuggled bustards and may cause sporadic disease outbreaks in captive collections.

Etiology

Chlamydophilosis is a multifactorial disorder in bustards resulting from a combination of nutritional, genetic, management, behavioral, and environmental factors, as well as infectious causes.

Chlamydophila psittaci has a direct life cycle in which birds exposed to clinical or subclinical shedders may develop the disease.

Transmission may occur by direct contact with the feces or with nasal and ocular discharges.

Clinical Signs and Findings

Clinical signs are highly variable and include depression, anorexia, ruffled plumage, conjunctivitis, oculonasal discharge, sinusitis, airsacculitis, diarrhea, biliverdinuria, coughing, and paralysis.

Disease is usually associated with low mortality in adults, but high mortality has been reported in juveniles.

Postmortem findings in bustards include sinusitis, fibropurulent tracheitis, pneumonia, airsacculitis, pericarditis, hepatitis, catarrhal enteritis, dry fibrinous peritonitis, pancreatitis, and splenomegaly.

Diagnostic Methods

Direct visualization in clinical specimens by staining impression smears.

Isolation of *C. psittaci* and identification.

Detection of a seroconversion and rising antibody titers.

Detection of antigens or genes.

Treatment

Houbara bustards are given weekly intramuscular or subcutaneous injections of doxycycline, 100 mg/kg, with maintained plasma levels in excess of 1 µg/mL, for 45 days.

Weekly injections of long-acting oxytetracycline at doses of 75 to 100 mg/kg have also been used effectively in bustards, but this agent is thought to cause local necrosis at the injection site as well as immunosuppression.

Doxycycline-impregnated feed pellets (1000 ppm) used during the quarantine period at the National Avian Research Center (NARC) have successfully treated chlamydophilosis in houbara bustards.

Chlamydophila is sensitive to heat and 1% formalin if the temperatures are above 20° C. Quaternary ammonium compounds and lipid solvents are poor choices for inactivating *Chlamydophila*. Benzalkonium chloride destroys infectivity within minutes.

Gram-negative bacilli are the predominant aerobic microflora of developing bustard chicks.²²

The most frequently reported bacterial diseases described in bustards include colisepticemia, mycobacteriosis, clostridiosis, salmonellosis, yersiniosis, pseudomoniasis, and erysipelothrix. Further information on these conditions is covered in depth by Bailey.² Box 22-2 presents the clinicopathologic features of chlamydophilosis in bustards.

Fungal Diseases

Aspergillosis was one of the first mycotic diseases to be described in animals, and *Aspergillus fumigatus* was first found in the lungs of a great bustard in 1863.¹⁵ Aspergillosis is common in smuggled bustards in the Middle East but occasionally occurs in captive-reared chicks. Aspergillosis has also been reported in captive and free-living great bustards.^{16,32}

Infection of the oropharyngeal cavity and esophagus in bustards by the yeast *Candida albicans* often results from antibiotic treatment. Infection occasionally occurs in juvenile bustards with an immature immune system but is more common in adult birds housed in unsanitary conditions.

Parasitic Diseases

Heavy tapeworm and roundworm infestations can result in morbidity in captive bustards. Mortality can occur in wild birds with heavy infestations that have been recently caught and are subjected to the stress of transportation. Parasites of both captive and free-living bustards have been studied extensively, but the effect of parasites on the population dynamics of free-living bustard populations has not been well documented.² Box 22-3 lists the helminth species identified in free-ranging and captive bustards. Vascular parasites, such as *Paronchocerca tonkinensis*, have been reported in buff-crested bustards²⁴ (Figure 22-10). The eye fluke *Philophthalmus distomatosa* (Trematoda; Philophthalmidae) has been found in the conjunctival sacs of white-bellied bustards with severe conjunctivitis.

In the Middle East, trichomoniasis is probably the most important protozoal disease of captive bustards (Figure 22-11). Other protozoa, however, including *Eimeria* spp., *Giardia* spp., *Histomonas* spp., and *Entamoeba anatis*, have also been reported as causative agents of digestive tract disease.³³

Box 22-3

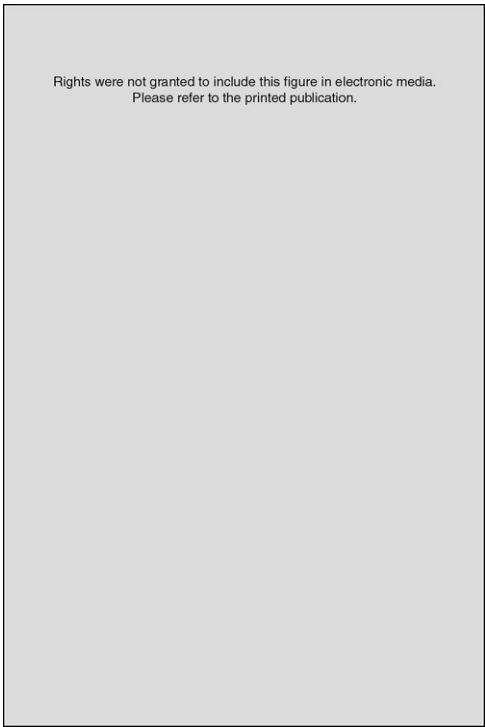
Helminth Species Reported in Bustards

Great Bustard Cestoda <i>Hispaniolepis</i> spp. <i>Raillietina cesticillus</i> Nematoda <i>Capillaria</i> spp. <i>Syngamus trachea</i> <i>Cyathostoma</i> spp. <i>Heterakis gallinae</i> Kori Bustard Cestoda <i>Otiditaenia conoides</i> <i>Otiditaenia</i> spp. <i>Ascometra chorioidis</i> <i>Ascometra scheuermani</i> <i>Parauterinidae</i> Acanthocephala <i>Mediorhynchus taeniatus</i> Nematoda <i>Capillaria</i> spp. <i>Syngamus trachea</i>	Houbara Bustard Cestoda <i>Hymenolepis ilosa</i> <i>Hispaniolepis falsata</i> <i>Raillietina neyrai</i> <i>Raillietina paroniella</i> <i>Otiditaenia conoides</i> <i>Otiditaenia macqueenii</i> <i>Idiogenes nana</i> <i>Idiogenes otidis</i> <i>Idiogenes</i> spp. <i>Ascometra chorioidis</i> <i>Ascometra vestita</i> Acanthocephala <i>Mediorhynchus taeniatus</i> <i>Centrorhynchus lancea</i> <i>Empodius taeniatus</i> <i>Sphaerirostris embae</i> Nematoda <i>Petrovifilaria mongolica</i> <i>Harteria rotunda</i> <i>Heterakis gallinarum</i> <i>Histiocephalus choriostidis</i>	<i>Subulura brumpti</i> <i>Paraspiralatus sakeri</i> <i>Capillaria</i> spp. <i>Ascaridia galli</i> Little Bustard Nematoda <i>Capillaria</i> spp. <i>Trichostrongylus</i> spp. Buff-Crested Bustard Cestoda <i>Raillietina neyrai</i> <i>Otiditaenia macqueenii</i> Nematoda <i>Allodapa</i> spp. <i>Paronchocerca tonkinensis</i>
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Data from Bailey TA, Wernery U, Howlett J, et al: *J Wildl Dis* 35:31-37, 1999.



Fig 22-10 *Paronchocerca tonkinensis* nematodes in right ventricle of buff-crested bustard. (Courtesy Phil Nicholls.)



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Fig 22-11 Large, caseous, trichomoniasis lesion in buccal cavity and proximal esophagus of a kori bustard. (See Color Plate 22-11.) (From *Association of Avian Veterinarians*: *J Avian Med Surg* 11:166-174, 1997.)

Noninfectious Diseases

Liver diseases, including amyloidosis, fatty liver, and hemosiderosis, are important conditions in bustards. Fatty liver disease in one group of houbara bustards was thought to be caused by high stocking density,



Fig 22-12 Recumbent houbara bustard with capture paresis. (See Color Plate 22-12.) (From Samour JH, editor: *Avian medicine*, London, 2000, Mosby.)



Fig 22-13 Bilateral distortion of legs of white-bellied bustard caused by bilateral rotation of tibiotarsal bones. (See Color Plate 22-13.)

poorly ventilated and dusty conditions, and poor nutrition.²³ Hemosiderosis and hemochromatosis have been reported in bustards. Excessive iron in the diet may cause hemosiderosis in other species, and diets for captive bustards should take these findings into account. Amyloidosis has been diagnosed in black, houbara, Heuglin's, and buff-crested bustards at NARC.

Musculoskeletal conditions, including capture paresis, are an important cause of morbidity and mortality of bustards during capture and translocation (Figure 22-12). Lameness is a common problem in many species of captive bustards.³ Developmental metatarsal deformities in growing chicks may make bustards susceptible to lameness as adults. Because of their rapid growth, bustard chicks are highly susceptible to developing long-bone, particularly leg, problems, including metabolic diseases, tibiotarsal rotation, chondrodystrophy, splayed legs, medial rotation of the phalanges, and slipped wing²¹ (Figure 22-13). Developmental abnormalities of the long bones often coincide with peak growth rates in the length of long bones, when the bones are susceptible to pathologic disturbances.

Other frequently observed conditions include the ingestion of foreign bodies in bustards maintained in outdoor aviaries and self-inflicted traumatic injuries in frightened captive bustards that fly into the side or roof of enclosures, particularly easily stressed and wild-derived individuals. Intraspecies trauma causes injuries and death in buff-crested, white-bellied, kori, and black bustards. Trauma caused by aggression between males, between females, and between males and females has been reported in great bustards.³

PHARMACEUTIC AGENTS

The use of pharmaceutical agents in bustards is not well studied. In general, the drugs used in this group are those used in other avian groups. Bailey and Samour⁵ present a list of drugs that have been used in bustards.

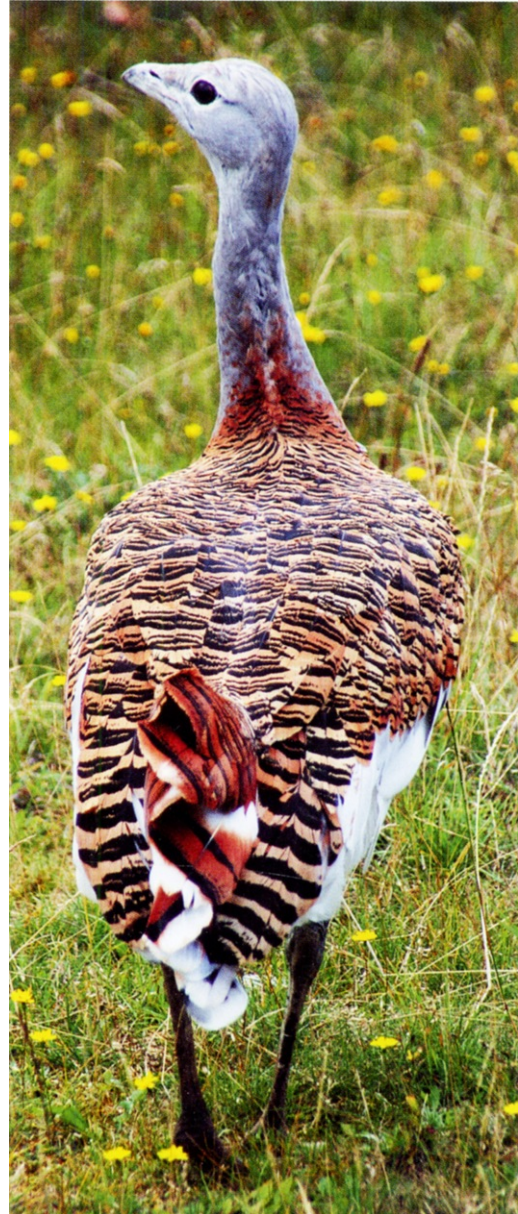
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Color Plate 22-1 Great bustard (*Otis tarda*) at the Great Bustard Trust, U.K. (For text mention, see Chapter 22, p. 171.) (Courtesy Thomas A. Bailey.)



Color Plate 22-2 Kori bustard (*Ardeotis kori*) in East Africa. (For text mention, see Chapter 22, p. 171.) (Courtesy Paul Goriup.)



Color Plate 22-4 In these naturalistic aviaries, pigeons and doves may be potential sources of disease for captive bustards. Bustards managed in captivity are exposed to diseases that they would not normally encounter in their natural environment. (For text mention, see Chapter 22, p. 173.)



Color Plate 22-9 Houdou bustard with Newcastle disease from paramyxovirus type 1 (PMV-1) infection, showing typical central nervous system signs of torticollis. (For text mention, see Chapter 22, p. 178.) (From *J Zoo Wildl Med* 28(3): 325-330, 1997.)



Color Plate 22-5 When restraining small to medium-sized bustards, the limbs can be tucked under the bird's body. (For text mention, see Chapter 22, p. 174.)



Color Plate 22-11 Large, caseous, trichomoniasis lesion in buccal cavity and proximal esophagus of a kori bustard. (For text mention, see Chapter 22, p. 182.) (From *Association of Avian Veterinarians: J Avian Med Surg* 11:166-174, 1997.)



Color Plate 22-6 A small cloth sack can be used to cover the head of medium-sized to large bustard species. It is important to have a hole so that the nares are not covered. (For text mention, see Chapter 22, p. 174.) (Courtesy Thomas A. Bailey.)



Color Plate 22-12 Recumbent houdou bustard with capture paresis. (For text mention, see Chapter 22, p. 183.) (From Samour JH, editor: *Avian medicine*, London, 2000, Mosby.)



Color Plate 22-13 Bilateral distortion of legs of white-bellied bustard caused by bilateral rotation of tibiotarsal bones. (For text mention, see Chapter 22, p. 183.)

CHAPTER 23

Medical Management of Curassows

MARYANNE E. TOCIDLOWSKI

The Houston Zoological Gardens (HZG) has been successful in raising and maintaining curassows in captivity, housing more than 230 curassows of 10 species since 1973. The information in this chapter is based on review of medical records, necropsy records, and personal zoo experience in dealing with this group of birds. The age of birds included in my study ranged from prehatchling to 31 years. Birds over 1 year of age were considered to be adults.

BIOLOGY

Curassows are a long-lived (20+ year), arboreal gallinaceous group of birds found in the Central and South American tropics and subtropics.² They are in the family Cracidae, a primitive bird group in the order Galliformes, and are distantly related and similar to grouse, quail, chicken, and other fowl species. The family Cracidae contains curassows (four genera, 14 species; Box 23-1), chachalacas, and guans.³ Curassows are important in seed dispersal, as environmental indicators, and for ecotourism. Several species of curassow are highly endangered or threatened in their environment as a result of habitat destruction and hunting.⁵

Natural enemies of curassows include predatory birds, mammals, and humans. Curassows are generally monogamous, usually occurring in pairs, although trios (cock and two hens) or family groups may be found existing peacefully together. The female lays and broods two eggs (occasionally only one or up to three), with incubation lasting approximately 28 to 30 days. Females may produce four clutches per year if the eggs are removed for artificial incubation or domestic chicken brooding after the clutch is laid. Chicks are precocial, grasping and perching as soon as they are hatched; thus smaller perching should be provided for the chick within 24 hours after hatching. The

chicks are fed by both parents by offering foods in their beak; curassow parents do not regurgitate for their young.

Curassows exhibit four interesting mannerisms for uncertain reasons, but believed to be for anxiety, nervousness, or courtship: (1) a rapid head flick from side to side, (2) the tail bob, (3) passing of the head over the back, such as a duck would do when preening, and (4) the wing flap. If one is not familiar with these behaviors, they could be misconstrued as neurologic conditions. It is thought that the head flick might have evolved as a defense to parasitic eye flies.¹

HOUSING

Curassows may be housed in a variety of ways depending on space limitations and general attitude of the bird. Curassow pens should be fairly large and well planted and should contain several perches of varying sizes for roosting because of the birds' body size and their arboreal nature. It is thought that curassows spend approximately half their time perching above the ground. An enclosed section for protection from the cold and frostbite should be included in all outdoor exhibits. Curassows are not cold tolerant and are susceptible to developing frostbite when the environmental temperatures fall below approximately 40° F (4.4° C) for several consecutive days.

Males may be territorial, and two or more housed together will tend to fight. The birds may be excessively aggressive during the breeding season and may even attack zoo visitors, as well as being aggressive toward smaller birds, sometimes killing them. Depending on their personality, some curassows are not a good species for free-flight pens, whereas other individuals do very well in open spaces and will cohabitate with other species.

UNIQUE ANATOMY

Curassows are fowl-like with strong legs and feet, ample tail and wings, and a well-developed hind toe used to grasp branches. Curassows are the only group of the Cracidae family that has a developed crop. They are primarily herbivorous, with a muscular gizzard that can grind hard seeds and nuts, as well as fruit, greens, insects, and invertebrates. Because of the muscularity of the ventriculus, curassows tend to swallow small pebbles or gravel as grit.

Male birds are usually larger than the females, and both genders are vocal with a well-developed syrinx. Certain species of male curassows, *Pauxi pauxi* and *Crax alector*, have an elongated trachea, used in vocalization for increased loudness or low-pitched sounds. In the northern-helmeted curassow (*Pauxi pauxi*) the trachea extends under the skin, overlays the abdomen, then curves back around and up and enters the thoracic inlet.³

Some curassow species have a feathered crest on the top of the head (*Crax* spp.) and a wattle or specialized knob or protuberance on the caudal cere. Males have an intromittent organ, which might be used to sex young birds. Gender determination of chicks of the *Crax* spp. may also be done by comparing cere color, which is pink or fleshy colored in males and dusky colored in females.

RESTRAINT AND TESTING

The curassows are large birds, weighing 2.5 to 3.5 kg (5.5-7.7 lb). They have large wings and strong feet with dangerous nails, so restraint of the legs and feet is important. Many minor procedures, such as physical examination, blood collection, medicating, and bandaging, may be done under manual restraint, which the birds seem to tolerate well. The suggested method of restraint is to force the bird to the ground by netting or cornering the bird. The legs are then secured in one hand, taking care to hold above the hocks with a finger between the legs. The wings are pinned against the bird's body and the head tucked under the arm so that it is facing away from the handler.⁷

Blood may be easily collected from the right jugular vein in most species. An obese bird might have too much adipose tissue in the neck to visualize the vein well. Other sites for blood collection are the ulnar/wing vein or the tarsus-metatarsus vein on the medial aspect of the leg. Table 23-1 provides the normal blood parameters of the wattled curassow.⁴ Because of the

Box 23-1

Curassow Taxonomy: Genus/Species (Common Name)*

Nothocrax urumutum (Nocturnal curassow)
Mitu tormentosa (Crestless curassow)
Mitu salvini (Salvin's curassow)
Mitu tubersum (Razor-billed curassow)
Mitu mitu (Alagoas curassow)
Pauxi pauxi (Northern-helmeted curassow)
Pauxi unicornis (Southern-helmeted curassow)
Crax rubra (Great curassow)
Crax alberti (Blue-billed curassow)
Crax daubentoni (Yellow-knobbed curassow)
Crax alector (Black curassow)
Crax globulosa (Wattled curassow)
Crax fasciolata (Bare-faced curassow)
Crax blumenbachii (Red-billed curassow)

Data from InfoNatura: Birds, mammals, and amphibians of Latin America [Web application], Arlington, Va, 2005, NatureServe.

*Genus and species vary slightly with different authorities.

long neck in curassows, it is preferred that oral medications, fluids, or food be given by a red rubber catheter or similar tubing, approximately 30 to 45 cm (12-18 inches) in length. This length is sufficient to ensure that the materials given are deposited in the crop. An adult curassow may take 60 mL of liquids orally by gavage although it is best to start with lower volumes in a sick bird and work up to larger volumes. Medication dosages given to curassows are those published in current avian formularies.

The HZG does not use injectable anesthetics for their birds. Isoflurane and oxygen inhalation anesthetic is given by mask, fashioned to fit the bird's face and knob. The nostrils lie just cranial to the knob or wattle on the featherless area of the caudal cere. Once anesthetized, the curassow may be intubated with a fairly large endotracheal tube (4-5 Fr), taking care not to fracture the bony protuberance in the center of the glottal opening to the trachea. Respiration and heart rate may be monitored with instruments designed for small mammals.

ANTEMORTEM DISEASES

Noninfectious Diseases

Curassows are generally healthy birds but, when found sick, often present with general clinical signs such as anorexia, abnormal behavior, depression,

Table 23-1

Representative Hematology and Plasma Biochemistry Values of Wattled Curassow (*Crax globulosa*)

Parameter	Units	ISIS			HZI		
		Mean	1 SD	N	Mean	1 SD	N
WBC	10 ³ /mL	21.9	15.6	73	19.6	14.2	40
RBC	10 ⁶ /mL	3.17	0.37	39	3.23	0.35	34
HGB	g/dL	15.2	2.6	39	15.2	2.0	34
HCT	%	43.4	6.2	73	42.1	4.3	41
MCV	fL	135.8	17.6	39	131.9	13.1	34
MCH	pg	47.1	5.3	34	47.4	5.3	34
MCHC	g/dL	36.1	4.7	38	36.0	3.8	34
Heterophils	10 ³ /mL	5.0	4.8	72	4.0	2.4	39
Lymphocytes	10 ³ /mL	14.2	13.6	72	11.7	11.6	40
Monocytes	10 ³ /mL	1.4	1.5	62	1.4	1.3	35
Eosinophils	10 ³ /mL	0.7	0.7	55	0.5	0.4	32
Basophils	10 ³ /mL	0.9	0.6	64	0.9	0.6	36
Plasma protein	g/dL	—	—	—	4.8	1.2	22
Glucose	mg/dL	296	43	71	309	47	36
BUN	mg/dL	3.0	1.0	48	3.5	0.9	31
Creatinine	mg/dL	0.3	0.1	11	0.3	0.1	10
Uric acid	mg/dL	9.8	3.2	46	9.8	3.6	35
Calcium	mg/dL	11.6	1.2	68	11.8	1.3	34
Phosphorus	mg/dL	6.7	2.0	31	6.0	2.7	3
Sodium	mEq/L	161	8	36	166	14	7
Potassium	mEq/L	3.8	1.2	36	4.1	1.6	7
Chloride	mEq/L	121	6	29	126	11	2
Iron	mg/dL	229	36	4	—	—	—
Cholesterol	mg/dL	178	34	53	170	31	32
Triglycerides	mg/dL	118	65	40	132	71	32
Total protein	g/dL	4.1	0.6	68	4.1	0.7	34
Albumin	g/dL	1.6	0.3	30	1.8	0.4	3
Globulin	g/dL	2.5	0.4	30	3.4	0.5	3
AST (SGOT)	IU/L	32	14	66	35	14	34
ALT (SGPT)	IU/L	13	7	47	14	7	30
Total bilirubin	mg/dL	0.3	0.2	31	0.3	0.1	19
ALP	IU/l	230	191	63	214	153	33
LDH	IU/L	851	846	19	1886	—	1
CPK	IU/L	1387	610	29	1180	312	5
CO ₂	mmol/L	13.7	3.2	6	—	—	—
GGT	IU/L	6	8	7	—	—	—

From International Species Information System (ISIS, Apple Valley, Minn) and Houston Zoological Gardens (HZG), Inc (HZI, Texas), in-house analysis.

SD, Standard deviation; N, number curassows tested; WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration; BUN, blood urea nitrogen; AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase (CK, creatine kinase); CO₂, carbon dioxide concentration; GGT, gamma-glutamyltransferase.

lethargy, weight loss, debilitation, emaciation, and lameness. Occasionally they are found moribund.

Noninfectious diseases predominated in curassows presented for medical attention at the HZG. Trauma was the problem most frequently diagnosed for the reasons of cage-mate aggression, incompatible neighboring species, self-mutilation (rubbing), parental trauma to young, and trauma during manual restraint. Fractures of toes, legs, and rarely wings and integument lacerations and tears were common. Some disfigurement or trauma necessitating amputation of a digit or phalanx occurred. Most of the musculoskeletal problems could be attributed to caging, restraint, and metabolic disease.

Many of the integument cases presented for medical care included lacerations (often from the male traumatizing the female), trauma to beaks and nails, and bumblefoot (caused by heavy body weight and poor perching). A frostbite-like syndrome has been found in several birds in Houston after the outdoor temperatures dropped to about 40° F for several days. Other, nontraumatic integument problems consisted of overgrown beak and nails and uropygeal impaction.

Curassows also have a tendency to pick up and ingest interesting objects off the ground, as well as normal pebbles and grit. There have been several cases of zinc toxicosis in captive zoo birds, with only rare reports of intestinal obstruction.

Reproductive problems found in curassow hens resulted from their large egg size and included eggshell retention, egg binding, soft-shelled eggs, and cloacal prolapse. Apparently, if a female had problems once, she was likely to have problems again. One bird was successfully hysterectomized for chronic egg binding. Intromittent organ prolapse and infection were seen in one adult male and resolved after various medical treatments.

Few diseases associated with the hematopoietic system were identified. One case was diagnosed as leukosis and another as severe anemia of unknown etiology. Other miscellaneous problems included ocular trauma and cataracts.

Infectious Diseases

In general, curassows are hardy birds and are not prone to disease. Because they are classified in the order Galliformes, however, it is believed that curassows are susceptible to most of the disease agents affecting poultry, such as *Salmonella* spp., *Mycoplasma* spp., *Chlamydophila* spp., and reticuloendothelial virus

(REV). A detailed list of diseases that affect gallinaeous birds is available in the literature.⁶

Very few infectious diseases have been diagnosed in live curassows at the HZG. Some birds have seroconverted to a suspicious status with *Salmonella* serology, but cultures have not been positive at this point. Nonspecific enteritis has occurred and responded well to antimicrobials. Bacterial pododermatitis has affected some birds, necessitating systemic and topical treatment with repeated bandaging. Low numbers of endoparasites have been found in the HZG birds and included ascarids, *Capillaria*, *Strongylus*, *Strongyloides*, *Dispharynx*, *Heterakis*, and coccidia. Feather lice and mites have also been found on several birds. Parasitic infestations responded well to anthelmintics and topical parasiticides.

Curassow Chicks

Many chicks at the HZG had problems caused by poor or abnormal hatching. Antemortem neonatal problems were associated mainly with retained or nonabsorbed yolks and complications of abnormal positioning in ovo. Curassow chicks older than 1 day generally have few problems. Rotational leg deformities have been found in chicks that did not have good perching material supplied after hatching.

POSTMORTEM FINDINGS

Diagnoses found at necropsies performed at the HZG reflected the clinical signs and diagnoses found in live birds. Forty-two complete necropsy reports that included histopathology were reviewed and the diagnoses recorded. Diseases affecting multiple organs were peritonitis (primarily in the hens from egg yolk contamination) and septicemia. Digestive tract diagnoses included enteritis, colitis, hemochromatosis, and hepatitis. Respiratory lesions included pneumonia and aspiration (especially in newly hatched or prehatched chicks), bronchitis, and aspergillosis. Aspergillosis was found in only four birds of the 42 records reviewed. Reproductive diseases of hens were similar to those in the live birds, such as egg binding, salpingitis, and metritis, with one ovarian granuloma found.

The HZG has seen necropsy lesions in several curassows that are typically found in birds infected with REV, such as lymphoid leukosis, lymphoma, and lymphoreticular disease. REV infection was not confirmed in any of these cases because most were

diagnosed histologically before REV polymerase chain reaction (PCR) testing was available. Other neoplasias found were intestinal carcinoma and adenocarcinoma.

Noninfectious gross necropsy lesions from birds that were euthanized because of poor prognosis or that had died included hypothermia, frostbite, trauma, and musculoskeletal deformities and malformations (rotational deformities, fractures, scoliosis, slipped tendons, bumblefoot, and myopathy). Few adult birds were presented for necropsy solely because of musculoskeletal problems, although one bird died shortly after fracture repair from a fat embolus.

Young birds were presented most often for euthanasia as a result of rotational deformities of the legs and slipped gastrocnemius tendons. Chicks and young birds also sustained fractures and trauma from parental trauma and neglect. Neonatal deaths were not well described, and in many cases no histologic lesions were found. Several chicks appeared to have died during the hatching process, some associated with malpositioning in the egg. Histologically, omphalitis, yolk sacculitis, pulmonary edema, and aspiration predominated.

CONCLUSION

Curassows are large, long-lived, unusual tropical gallinaceous birds that are not difficult to keep and

maintain in a zoologic setting. Curassow species are susceptible to cold stress and disease, but with good quarantine, disease-screening protocols, and husbandry procedures, most diseases may be prevented or treated.

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CHAPTER 24

Monitoring Avian Health in the Galápagos Islands: Current Knowledge

LUIS R. PADILLA AND PATRICIA G. PARKER

The Galápagos Archipelago is located in the Pacific Ocean, approximately 1000 km (600 miles) west of the South American coast at the level of the equator. The islands and associated islets are a part of Ecuador (Figure 24-1). “Galápagos” refers to the Spanish name given to the giant land tortoises native to the islands. Beginning with Charles Darwin’s visit in 1835, several studies of this unique reservoir of endemic biodiversity have formed an important part of the foundation of modern evolutionary theory. At least 26 endemic land bird species are present on the islands, including the 13 highly diversified finch species often referred to as “Darwin’s finches.”

Permanent human colonization of the Galápagos Islands was delayed until the 1800s, but significant anthropogenic events have occurred in the last two centuries, including the introduction of foreign species, environmental change by humans and introduced species, harvesting of wildlife, and expanding human settlements. Since 1959, the islands have been protected as the Galápagos National Park, and the surrounding waters have been protected as the Galápagos Marine Reserve since 1998. Human settlement is currently limited to less than 3% of the land, with the majority of the people living on Santa Cruz and San Cristóbal, followed by small human populations on Isabela and Floreana. However, the resident human population has continued to grow rapidly, and the introduction of exotic, sometimes invasive, species continues to be a threat despite aggressive efforts to exclude them.²⁴

The potential introduction of new pathogens has been a growing concern to the conservation of Galápagos birds.²⁹ Pathogens may have severe effects when introduced to novel ecosystems, where individuals may lack natural resistance, and island populations might be particularly susceptible. For example,

diseases, especially avian pox and avian malaria, have been implicated as major factors in the decline of native avian populations in the Hawaiian Islands.^{21,22,30} Unlike Hawaii, the Galápagos avifauna has undergone relatively few extinctions, and no bird species has become extinct. In the 1980s, *Culex quinquefasciatus*, the mosquito vector for avian malaria, was identified in Galápagos, and its establishment was recently confirmed.²⁸ The observation of avian poxlike lesions noticed in domestic chickens on the Galápagos Islands, followed by similar observations often made in endemic birds, raised significant local concerns about the possible introduction of pathogens to the endemic populations.

Recognizing the need for gathering objective information to assess the risk of introduced diseases to the archipelago, a collaborative effort was initiated in 2001 among the Galápagos National Park Service, the Charles Darwin Research Station, the University of Missouri–St. Louis (UMSL), and the St. Louis Zoo.¹³ The major goals were to establish current health status of native bird populations and to identify disease threats. Additional goals included conducting research studies to elucidate mechanisms and evolution of disease in island populations and to facilitate the transfer of knowledge among partners through training opportunities and workshops.

This chapter summarizes the current state of knowledge on this ongoing effort to monitor avian health in the Galápagos Islands. The efforts of us and our associates may be broadly classified in several categories: general health assessment of bird populations, identification and characterization of known pathogens, and identification of avian pathogens in domestic chickens. Ultimately, the information gathered as part of these conservation efforts should be applicable to the management and conservation of the Galápagos Archipelago’s unique avifauna.



Fig 24-1 The islands and islets of the Galápagos are a part of Ecuador.

LOGISTICS OF FIELD STUDIES IN REMOTE OCEANIC ISLANDS

The Galápagos health studies have been possible because of a unique relationship with ecologists and evolutionary biologists. Partnerships with basic researchers have allowed us to minimize the handling efforts, obtain access, and minimize duplication of resources needed to access wild bird populations throughout the archipelago. In most instances, this relationship has allowed us to interpret the roles of health, disease, host, and pathogen within an ecologic context that is applicable to species management and conservation at the population level.²⁶

All the studies were conducted in strict accordance with guidelines stipulated by the Galápagos National Park and in adherence to preobtained permits. Most uninhabited islands in the Galápagos Archipelago pose unique logistical challenges to access, collection, and preservation of biologic samples.

Whole blood collected for genetic studies has been preserved in a lysis-buffered solution,⁸ which maintains genetic material stable through a broad range of environmental temperatures without the need for refrigeration. We have used a field centrifuge unit to harvest serum or plasma in the field and therefore minimize artifacts related to red blood cell metabo-

lism. Field centrifuge units are available from several manufacturers (e.g., Vulcon Technologies, Grandview, Missouri) and can be powered from a battery source. Fresh blood smears have been prepared immediately after collection to minimize risk of artifacts, and sometimes these have been fixed and stained in the field using commercially available, modified Wright-Giemsa stains. We have also obtained adequate quality smears without fixing them in the field. White blood cell (WBC) counts have subsequently been done on whole blood using standard avian WBC count techniques with a hemacytometer and a field microscope. Alternatively, for small blood samples, and when fresh whole blood was not immediately available, leukocyte counts have been estimated from fixed blood smears using standard techniques. In cases in which a microscope was not immediately available (or impractical to take in the field), the smear estimation technique has been shown to provide reliable and practical leukocyte counts.¹⁷ Samples that were stored frozen were usually transferred to Nalgene cryotubes, which may withstand the low temperatures of cryostorage.

Biologic samples (e.g., serum, plasma, tissues, swabs) have been frozen in the field using a portable cryopreservation unit ("dry shipper"). These units must be precharged with liquid nitrogen, but once charged, will hold the temperature and preserve samples for a

prolonged period, depending on the unit specifications. The design of the dry-shipper unit is a double wall that absorbs the liquid nitrogen, and the vapors in the sample-holding chamber maintain the samples frozen. Thus there is no standing liquid nitrogen, making these units safe, light, and practical for field use. In addition, these units may be transported safely in most commercial airlines if properly loaded. When thawing samples, we have learned that extreme caution must be taken in the case of inadequate sample containers, because these may expand rapidly as temperatures equilibrate and may expel contents at high speeds, with the risk of significant bodily injury.

International, national, and local permits from agencies regulating wildlife, agricultural, or biologic trade have always been obtained before samples were transported to the appropriate laboratories for testing.

GENERAL HEALTH ASSESSMENT OF BIRD POPULATIONS

Health assessment studies provide a baseline of information against which health-related threats may be compared, including establishing reference parameters for normal hematology and plasma chemistry values and identifying serologic exposure to potential pathogens in natural populations. Species systematically surveyed were studied concurrently with ecologic or population surveys. These combined studies provided a natural history background against which the information gathered could be interpreted. Colonial and aquatic species are useful subjects for these studies because their health is likely to reflect the status of their ecosystems. Colonial living habits could make certain species more susceptible to epizootics and to both natural and anthropogenic environmental disasters.

Significant Findings to Date

The waved albatross (*Phoebastria irrorata*) is a well-known member of the Galápagos avifauna, and population estimates suggest that a minimum of 31,000 to 35,000 adults exist.¹ The majority of the population breeds on the island of Española, in the Galápagos Archipelago, but a few pairs may breed on the island of La Plata, near the west coast of Ecuador. The Galápagos population has been stable for at least 25 years, but it may be considered "vulnerable" by criteria set by the International Union for Conservation

of Nature and Natural Resources (IUCN), on the basis of a restricted breeding range. As part of a health assessment study, hematologic and plasma biochemistry reference values were established for this species (Table 24-1), and a serologic survey showed no seroreactivity to the majority of common avian pathogens, with the exception of common seroreactivity to group-1 adenoviruses.¹⁴

The flightless cormorant (*Phalacrocorax harrisi*) is an endemic species only found in the western part of the Galápagos Archipelago, on Fernandina and Isabela islands. They were studied throughout their geographically limited range. Hematology assessments, plasma biochemistries, and microscopic evaluation of blood smears revealed the frequent occurrence of microfilariae (33.3%) in this species.¹⁹ Although seronegative to a broad panel of avian pathogens (including paramyxoviruses 1, 2, and 3), seroreactivity to group-1 adenoviruses was a common finding (30.9%) in the sampled population, which was similar to observations made for the waved albatross. Seroreactivity to *Chlamydophila psittaci* was seen in 10.8% of the cormorants sampled.

A concurrent study done on the endangered Galápagos penguin (*Spheniscus mendiculus*), another endemic species, also detected circulating microfilariae in a large percentage (>20%) of blood smears collected between 2003 and 2004.²⁰ Birds were seronegative to a broad panel of infectious diseases, but a large percentage of the penguins showed seroreactivity to *C. psittaci*, indicating that exposure might be common in this species.²⁰

On Genovesa Island, located in the northeastern part of the archipelago, four species of seabirds—red-footed boobies (*Sula sula*), great frigatebirds (*Fregata minor*), Nazca boobies (*Sula granti*), and swallow-tailed gulls (*Creagrurus furcatus*)—were studied from the same colony in 2003.¹⁶ In addition to establishing baseline reference values for these species (Table 24-1), birds were specifically tested for *C. psittaci* and screened for hemoparasitism. Although no evidence of *C. psittaci* was detected, we observed low-grade, circulating parasitemias by *Haemoproteus*-like organisms in three of the species, with varying frequencies. Prevalences were highest in great frigatebirds (29.2%; 7/24), followed by swallow-tailed gulls (15.8%; 3/19), red-footed boobies (8.7%; 2/23), and Nazca boobies (0/25). The clinical significance and disease consequences of these hemoparasites are still unknown, but infected great frigatebirds showed higher heterophil:lymphocyte ratios than uninfected birds. This ratio has been proposed as a reliable indicator of stress in domestic chickens.⁶

Table 24-1

Hematology and Plasma Biochemistry Reference Ranges for Five Species of Free-Ranging Galápagos Birds

	Waved Albatross (<i>Phoebastria irrorata</i>) ¹⁴	Red-Footed Booby (<i>Sula sula</i>) ¹⁶	Nazca Booby (<i>Sula granti</i>) ¹⁶	Great Frigatebird (<i>Fregata minor</i>) ¹⁶	Swallow-Tailed Gull (<i>Creagrus furcatus</i>) ¹⁶
White blood cell count ($\times 10^3/\mu\text{L}$)	5.9 \pm 2.4	10.3 \pm 4.7	9.4 \pm 3.5	7.5 \pm 2.7	4.8 \pm 2.7
Heterophils (%)	66.1 \pm 30	36.1 \pm 16.7	46.7 \pm 14.3	39.0 \pm 8.5	54.0 \pm 11.1
Monocytes (%)	1.7 \pm 1.7	3.7 \pm 2.6	3.8 \pm 2.2	2.5 \pm 1.6	4.9 \pm 4.2
Lymphocytes (%)	30.5 \pm 15	54.8 \pm 17.5	34.4 \pm 14.2	40.0 \pm 12.0	36.2 \pm 10.9
Eosinophils (%)	1.7 \pm 1.7	5.2 \pm 3.6	15.4 \pm 7.9	17.9 \pm 2.7	4.4 \pm 1.8
Basophils (%)	0.0 \pm 0.01	0.2 \pm 0.4	0.5 \pm 0.7	0.6 \pm 0.9	0.5 \pm 0.8
Hematocrit (%)	38 \pm 5	50 \pm 7	51 \pm 3	55 \pm 8	54 \pm 5
Total protein (g/dL)	4.5 \pm 0.6	3.56 \pm 0.3	3.76 \pm 0.4	3.58 \pm 0.5	4.13 \pm 0.8
Albumin (g/dL)	1.8 \pm 0.2	1.17 \pm 0.1	1.22 \pm 0.1	0.94 \pm 0.1	1.57 \pm 0.4
Globulin (g/dL)	2.8 \pm 0.5	2.0 \pm 0.9	2.4 \pm 0.6	2.6 \pm 0.4	1.8 \pm 1.3
Phosphorus (mg/dL)	3.4 \pm 0.8	10.8 \pm 4.6	5.6 \pm 2.6	4.7 \pm 1.5	4.6 \pm 4.9
Calcium (mg/dL)	9.8 \pm 1.1	9.3 \pm 0.7	9.5 \pm 0.64	9.1 \pm 0.66	11.0 \pm 2.9
Glucose (mg/dL)	229.4 \pm 35.4	180.5 \pm 64.0	252.0 \pm 34.6	212.1 \pm 45.7	280.6 \pm 31.8
Sodium (mEq/L)	152.7 \pm 6.2	151.4 \pm 3.7	154.5 \pm 3.6	145.4 \pm 8.2	155.0 \pm 9.2
Potassium (mEq/L)	3.7 \pm 0.8	6.6 \pm 2.1	3.0 \pm 1.2	3.0 \pm 1.6	3.0 \pm 0.7
Chloride (mEq/L)	118.0 \pm 7.7	116.0 \pm 4.7	117.9 \pm 3.2	114.6 \pm 4.5	122.2 \pm 7.5
Uric acid (mg/dl)	4.4 \pm 2.7	10.9 \pm 8.2	12.1 \pm 7.2	7.7 \pm 7.7	7.5 \pm 5.9
Aspartate transaminase (U/L)	117.6 \pm 46.9	465.8 \pm 181.1	310.3 \pm 141.7	248.1 \pm 95.1	361.6 \pm 172.2
Creatine kinase (U/L)	290.0 \pm 173.1	940.1 \pm 371.4	871.8 \pm 271.1	556 \pm 421	263.3 \pm 323.1

CHARACTERIZATION OF PATHOGENS

Pathogens of Concern to Galápagos Columbiformes

The rock pigeon or domestic pigeon, *Columba livia*, is a recent anthropogenic introduction to the Galápagos Islands, and populations have inhabited Santa Cruz, San Cristóbal, and Isabela islands. Although major eradication efforts are under way, this species has been a source or concern for the introduction of certain pathogens to the endemic Galápagos dove (*Zenaida galapagoensis*). No other columbiform species is present in the archipelago. A study established the occurrence of common columbiform pathogens (*Trichomonas gallinae*, *C. psittaci*, *Haemoproteus* spp., hemoparasites, and *Salmonella* spp.) in the two species.¹⁵ *Trichomonas gallinae* was reported in both species in only those islands where the species coexisted.⁷ *Trichomonas gallinae*

was detected in 44% of rock pigeons from the human-inhabited island of San Cristóbal, but not in any endemic Galápagos doves. *Chlamydophila psittaci* was found only on Galápagos doves from Española Island. A near-ubiquitous sample prevalence (89%) of a *Haemoproteus*-like blood parasite was seen in *Z. galapagoensis* from all the islands but was not detected in rock pigeons.¹⁵

Avian Poxviruses in the Galápagos Islands

Lesions consistent with a poxlike virus have been described in several species of endemic Galápagos birds,¹⁸ including several species of Galápagos finches (*Geospiza*, *Camarhynchus*), yellow warblers (*Dendroica petechiae*), Galápagos mockingbirds (*Nesomimus parvulus*), and Galápagos doves (*Z. galapagoensis*). Pox-associated

mortality has been documented in Galápagos mockingbirds²³ but is likely to occur in all species and to worsen with El Niño events. Histopathology of cutaneous lesions from opportunistically sampled ground finches (*Geospiza* spp.) and yellow warblers revealed inclusion bodies diagnostic of *Avipoxvirus* spp., and similar lesions have been confirmed in domestic chickens on Galápagos.⁵ Subsequent molecular characterization of the poxvirus in endemic passerines showed two canarypox virus strains, whereas the domestic chickens on the islands are infected with the distinct fowlpox virus,¹⁸ objectively dispelling local concerns that an avipoxvirus from introduced chickens could have mutated into endemic wild birds.

Parasites Documented in the Galápagos Islands

It is generally believed that island populations carry lower parasite diversity than their mainland counterparts,³ and studying the dynamics of host-parasite interactions and phylogenetic relationships may help understand the evolution of disease and disease-avoidance trends of hosts. As part of multiple efforts (active, systematic surveillance, surveillance of necropsy records), parasites have been documented in a large number of Galápagos species. Table 24-2 lists the parasites that have been documented in Galápagos birds. A large number of previously unidentified parasites have been recovered. Further studies are currently under way, so we have used the terms “undescribed” for possible new species and “unidentified” for those that we classified only to the family or genus level at the time of this writing.

Blood Parasites

Nematodes. An unidentified nematode filarid was frequently identified in flightless cormorants and Galápagos penguins. It appears that the same nematode is present in both species, although no clinical signs have yet been identified in association with this parasite. We are pursuing genetic studies of these nematodes.

Apicomplexan Blood Parasites. *Haemoproteus*-like parasites were observed in peripheral blood smears from three of four species of Genovesa Island seabirds (two endemic), as well as in the sympatric and endemic Galápagos dove. Most Galápagos doves sampled have significant numbers of a *Haemoproteus*-like parasite detectable on peripheral blood smears, with prevalence ranging from 42% to greater than 96%

on different islands.^{15,16} The *Haemoproteus* hemoparasites have traditionally been considered incidental and relatively nonpathogenic vertebrate parasites, although effects on host fitness^{9,12} have been suggested, and pathogenicity has been documented for some species.⁴ No pathologic effects of *Haemoproteus*-like organisms have been documented in any Galápagos species. The highly pathogenic strains of *Plasmodium* associated with avian malaria have not been documented in the Galápagos Islands.

Kinetoplast Blood Parasites. An unidentified *Trypanosoma* sp. parasite was recovered on a peripheral blood smear from a Galápagos hawk on Santiago Island. We sequenced a small region of the *ssu* rDNA gene from bird-blood-derived DNA, and a BLAST search on Genbank showed that this species is closely related to other raptor-derived *Trypanosoma* spp., although its distribution or prevalence is unknown.

Ectoparasites

Four endemic birds have been specifically sampled for ectoparasites on 18 island populations.^{25,27} Acari have been recovered from families of Epidermoptidae and Argasidae; lice of genera *Pectinopygus*, *Piagetialla*, *Colpocephalum*, *Degeeriella*, *Craspedorhynchus*, *Columbicola*, and *Physconelloides*; and Hippoboscid flies of genera *Olfersia*, *Icosta*, and *Microlynchia*, as well as undescribed lice and unidentified mites (see Table 24-2). Opportunistic samples from individuals of other species than these focal four have recovered unidentified mites and lice from *Brueelia* and *Myrsidea* genera.

An obligate dipterian bird parasite, *Philornis downsi*, was first detected as a parasite of finch nestlings in the Galápagos Islands in 1997 and appears to be ubiquitous on nests surveyed on Santa Cruz Island.² Although adult flies are nonparasitic, the larvae are obligate bird parasites. *P. downsi* typically affects nestlings and has been documented in nests from several finch species, Galápagos mockingbird, dark-billed cuckoo, yellow warbler, and vermilion flycatcher. Another parasite, *Sarcodexia lambens*, has been known to parasitize Galápagos finches, but this is a non-specific parasite that affects multiple taxa.²

Surveillance of Avian Pathogens in Domestic Chickens

Introduced species carry the risk of foreign pathogens being introduced into native island populations. Several bird species have been purposefully introduced

Table 24-2

Summary of Avian Parasites and Host Species Documented in the Galápagos Islands

Species	Ectoparasites	Endoparasites	Island
Waved albatross (<i>Phoebastria irrorata</i>)	<i>Ornithodoros</i> spp. (Argasidae)		Española
Flightless cormorant (<i>Phalacrocorax harrisi</i>)	<i>Pectinopygus</i> spp. (Phthiraptera); <i>Olfersia sordida</i> (Hippoboscidae); <i>Myialges caulotoon</i> (Epidermoptidae)	Undescribed microfilariae (Nematoda)	Fernandina, Isabela
Brown pelican (<i>Pelecanus occidentalis</i>)	<i>Piagetialla</i> spp. (Phthiraptera)	<i>Renicola</i> spp. (Trematoda); <i>Contracecum</i> spp. (Nematoda)	Santa Cruz
Blue-footed booby (<i>Sula nebouxii</i>)		<i>Renicola</i> spp. (Trematoda); <i>Contracecum</i> spp. (Nematoda)	
Red-footed booby (<i>Sula sula</i>)		Undescribed <i>Haemoproteus</i> sp. (Haemoproteidae)	Genovesa
Great frigatebird (<i>Fregata minor</i>)		Undescribed <i>Haemoproteus</i> sp. (Haemoproteidae)	Genovesa
Swallow-tailed gull (<i>Creagrus furcatus</i>)		Undescribed <i>Haemoproteus</i> sp. (Haemoproteidae)	Genovesa
Galápagos penguin (<i>Spheniscus mendiculus</i>)	Undescribed louse (Phthiraptera)	Undescribed microfilariae (Nematoda)	Fernandina, Isabela
Galápagos hawk (<i>Buteo galapagoensis</i>)	<i>Colpocephalum turbinatum</i> ; <i>Degeeriella regalis</i> ; undescribed <i>Craspedorhynchus</i> spp. (Phthiraptera); <i>Icosta nigra</i> (Hippoboscidae); <i>Myiagles caulotoon</i> (Epidermoptidae) ²⁶	Undescribed <i>Trypanosoma</i> sp. (Kinetoplastidae)	Española, Fernandina, Isabela, Marchena, Pinta, Santa Fe, Santiago
Galápagos dove (<i>Zenaida galapagoensis</i>)	<i>Columbicola macrourae</i> , <i>Physconelloides galapagensis</i> (Phthiraptera); <i>Microlynchia galapageonsis</i> (Hippoboscidae); unidentified feather mite (Astigmata) ²⁷	Undescribed <i>Haemoproteus</i> sp. (Haemoproteidae); <i>Trichomonas gallinae</i> ⁷ ; <i>Eimeria palumbi</i> (Eimeriidae) ¹¹	Española, Genovesa, San Cristóbal, Santa Cruz, Santa Fe, Santiago
Medium ground finch (<i>Geospiza fortis</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Small ground finch (<i>Geospiza fuliginosa</i>)	<i>Philornis downsi</i> (Muscidae) ²	Unidentified coccidian	Santa Cruz
Vegetarian finch (<i>Camarhynchus crassirostris</i>)	Unidentified mites (Acari)		Santa Cruz
Cactus finch (<i>Geospiza candens</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Large tree finch (<i>Camarhynchus psittacula</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Small tree finch (<i>Camarhynchus parvulus</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Woodpecker finch (<i>Cactospiza pallida</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz

Table 24-2—cont'd

Summary of Avian Parasites and Host Species Documented in the Galápagos Islands

Species	Ectoparasites	Endoparasites	Island
Warbler finch (<i>Certhidea olivacea</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Yellow warbler (<i>Dendroica petechiae</i>)	<i>Philornis downsi</i> (Muscidae) ²	<i>Contracecum</i> spp. (Nematoda); unidentified coccidian	Santa Cruz, Genovesa, Santa Cruz
Vermilion flycatcher (<i>Pyrocephalus rubinus</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Galápagos mockingbird (<i>Nesomimus parvulus</i>)	<i>Brueelia</i> spp., <i>Myrsidea</i> spp. (Phthiraptera) <i>Philornis downsi</i> (Muscidae) ²	Unidentified protozoan causing systemic infection; unidentified coccidian <i>Polysporella</i> <i>genovesae</i> (Eimeriidae) ¹⁰	Fernandina, Genovesa, Marchena, Pinta, Santa Cruz, Santa Fe
Hood mockingbird (<i>Nesomimus macdonaldi</i>)	<i>Brueelia</i> spp., <i>Myrsidea</i> spp. (Phthiraptera)		Española
Dark-billed cuckoo (<i>Coccyzus melacoryptus</i>)	<i>Philornis downsi</i> (Muscidae) ²		Santa Cruz
Smooth-billed ani (<i>Crotophaga ani</i>)	<i>Philornis downsi</i> (Muscidae) ²		
Domestic chicken (<i>Gallus gallus</i>)	Unidentified lice (Phthiraptera); <i>Epidermoptes</i> <i>bilobatus</i> (Epidermoptidae)	<i>Oxyspirura mansonii</i> , <i>Capillaria</i> spp., <i>Dispharynx</i> spp., <i>Tetrameres</i> spp., <i>Ascarida galli</i> , <i>Heterakis gallinae</i> (Nematoda); <i>Raillietina</i> <i>echinobothrida</i> , <i>Davaina proglottina</i> (Cestoda) Unidentified renal trematodes, intestinal flagellates, and enteric coccidians <i>Toxoplasma gondii</i> ⁵	Santa Cruz, San Cristóbal
Rock dove (<i>Columba livia</i>)		<i>Trichomonas</i> <i>gallinae</i> ¹⁵	Santa Cruz, San Cristóbal

to the islands as farm animals: domestic chickens (*Gallus gallus*), domesticated turkeys (*Meleagris* spp.), guinea fowl (*Numida meleagris*), and domestic ducks (*Anas* and *Cairina* spp.). Other bird species have been introduced by humans for purposes other than domestication, such as rock pigeons (*Columba livia*), and smooth-billed anis (*Crotophaga ani*). In addition, domestic chicken production has increased in recent years in response to demand from a booming human

population and growing tourist industry in the islands.⁵ Galápagos law and quarantine regulations limit importation of domestic chickens to healthy birds originating from approved aviculture facilities in continental Ecuador, and vaccination is tightly regulated or prohibited. Chickens are present around human settlements on Santa Cruz, San Cristóbal, Isabela, and Floreana, and feral chicken populations exist on some of these islands.⁵

General surveillance of disease in chicken farms identified Newcastle disease (paramyxovirus type 1, PMV-1), *Mycoplasma gallisepticum*, and proventricular parasites as potential threats from chickens to endemic bird populations,⁵ although additional studies are currently under way to characterize the risk further based on type of farming operation and geographic location. In addition, a large number of chickens showed seropositivity to infectious bursal disease, group-1 avian adenoviruses, Marek's disease, avian encephalomyelitis, and two strains of infectious bronchitis virus. In addition to disease agents, some concern exists regarding the disposition of litter from production operations, which may significantly affect the health of local ecosystems.

TRAINING PROGRAMS

In conjunction with disease surveillance efforts, training and capability building have been major goals of our collaborations. Through the partnership of the St. Louis Zoo, the University of Missouri—St. Louis, Galápagos National Park, and Charles Darwin Research Station, the scientific endeavors have provided a forum for cultural and academic exchange among participants. Two training workshops have been conducted in the Galápagos Islands to disseminate information on avian diseases of interest and avian necropsy and phlebotomy techniques. Participants in these workshops have included local veterinarians, quarantine agents, field biologists, and park personnel. In addition, a full-time veterinary pathologist stationed at the Charles Darwin Research Station, on Puerto Ayora, Santa Cruz, is available as a diagnostic and educational resource to local biologists and veterinarians.

SIGNIFICANCE OF STUDIES AND CONSERVATION IMPLICATIONS

These studies have established a baseline of information on pathogens present in wild bird populations and have provided a set of reference ranges for healthy birds against which future disease concerns may be gauged. Some of these studies have interesting ecologic and evolutionary implications and provide new information for understanding host-pathogen dynamics and the evolution of these relationships in island populations. The information gained through surveillance of domestic-poultry pathogens will allow proper risk assessment and will provide objective data for management and conservation of the Galápagos

Islands in ways that balance wildlife preservation with economic sustainability. Additional work is under way to further characterize wild bird hemoparasites, further identify parasites of specific hosts, and continue disease surveillance through mortalities presented to the pathologist stationed at the Charles Darwin Research Station.

At least one study has suggested a link among island size, genetic diversity, and innate natural antibody responses, which may lead to lower antibody levels on small islands with lower genetic diversity and higher susceptibility to parasites.²⁶ This would suggest that small island populations, with limited innate natural antibody capabilities, might need more protection from introduced pathogens. Efforts to integrate health studies with ecologic and evolutionary research will continue, with the goal of providing a solid framework for objective assessment of disease risks to Galápagos bird populations.

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Avian Atherosclerosis

JUDY ST. LEGER

Vascular pathology in avian species includes a spectrum of lesions and etiologies similar to conditions in mammals. Of these conditions, atherosclerosis is the most common vascular change across the class Aves.

Arteriosclerosis is a loss of arterial elasticity caused by intimal thickening. This thickening results from migration of smooth muscle cells from the tunica media and increased mitotic division of these cells. Intimal expansion occurs from elaboration of increased extracellular matrix (ECM).¹⁸ Arteriosclerotic plaques of varying size, composed primarily of fibrous tissue between the intima and internal elastic lamina, are common findings in many birds. These plaques can form in chickens as young as 4 weeks of age. The plaques are appreciated grossly as pale areas of increased thickening of the intima, most often in the aorta and major arteries.¹⁵ These plaques may extend as circumferential lesions with marked luminal narrowing (Figure 25-1).

Atherosclerosis is a condition of arteriosclerosis associated with additional intimal accumulations of foamy cells, extracellular lipid, cholesterol, and mineralization. In birds, cartilaginous or osseous metaplasia can be seen in these lesions, as well as osseous metaplasia. Macrophages and other leukocytes are variably present. Vascular changes often involve both the intima and the media, with disruption of the internal elastic lamina. The term *atherosclerosis* is derived from the Greek words for “gruel” (*athero*) and “hardening” (*sclerosis*). The reference to gruel refers to the soft core of lipid and variable necrosis in these lesions.

Atherosclerotic plaques are composed of collagen and proteoglycans. Smooth muscle cells produce the ECM of the plaques. After 6 weeks of a cholesterol-supplemented diet, Japanese quail with a propensity to develop atherosclerosis have elevated glycosaminoglycan levels and foam cell development in atheromatous plaques. These foam cells disrupt the collagen ECM. After 10 weeks on a cholesterol-supplemented diet, there is disorganization in aortic intimal collagen fibrils. Total vascular collagen does not necessarily increase.²⁵

In humans, atherosclerotic changes progress in a described sequence, with generalized relationships to the patient’s age and clinical condition. Lesions begin as intimal fatty streaks, and early expansion is by lipid accumulation. These early lesions may begin in the patient’s teenage years. Smooth muscle and collagen proliferation accelerate about the fourth decade of life, resulting in formation of fibroatheromas, often with areas of central necrosis and mineral deposition. These changes may advance to the clinical phase of vascular disease, with progressive vascular stenosis, vascular thrombosis, and vascular degeneration with aneurysmal dilation or rupture, or both.

Atherosclerotic lesions in birds occur at the great vessels at the base of the heart and within the heart itself (Figure 25-2). The most frequently affected site is the aorta at the heart’s base. Other sites of importance include the brachiocephalic trunk, pulmonary artery, dorsal aorta, heart valves, and mural arteries. Aortic atherosclerosis in penguins may variably extend along the aorta to the level of the renal arteries. In all cases, lesions are more pronounced at the level of, or just before, the branching of smaller arteries. Unlike the condition in humans, associated aneurysmal dilation is not common in birds, except turkeys.

Turkeys are also the exception that makes the rule for lesion distribution of this condition. The muscular abdominal aorta of turkeys is much more susceptible to atherosclerosis than the elastic thoracic portion. In a study of 157 wild male turkeys collected by hunters in the early 1980s, birds demonstrated lesions most frequently in the sciatic arteries. Other affected sites, in order of decreasing frequency, were the aorta at the celiac region, cranial abdominal aorta, aorta at the sciatic bifurcation, caudal abdominal aorta, coronary arteries, and finally, thoracic aorta.¹⁷ This dramatically different lesion distribution suggests a possibly different etiology compared with other avian species. In quail, etiologic conditions responsible for atherosclerosis of the abdominal aorta are different than those resulting in lesions in the coronary arteries.



Fig 25-1 Photomicrograph of the aorta from aged Amazon parrot. Note the luminal compromise caused by marked thickening of the wall. (See Color Plate 25-1.)



Fig 25-2 Diffuse and multifocal thickening of vessels at base of the heart in aged Amazon parrot. (See Color Plate 25-2.)

Relationships of atherosclerosis to age and gender in birds are not as clear-cut as in humans. Atherosclerosis is generally a condition of adults. In spontaneous avian atherosclerosis, although lesions have been reported in a bird less than 1 year of age, most affected birds are older adults, with severity increasing with age.² Gender predilections have varied from report to report. Some studies demonstrate a male bias,¹¹ some a female bias,¹⁰ and some no gender bias at all.² In a review of 57 cases of atherosclerosis in seven species of penguins at all SeaWorld parks, there was no gender

predilection. Lesions were limited to animals older than 4 years, and severity did increase with increasing age within species.

Chronic conditions of geriatric animals become increasingly common as avian health and nutrition improve. Atherosclerosis is a condition of interest because it is common in adult birds and may progress to fatal lesions. Additionally, similarities between avian and human atherosclerosis have promoted the use of various avian species as models for human pathogenesis and management.

CLINICAL SIGNS

Clinical signs of avian atherosclerosis are referable to vascular disease. As the vascular changes progress, there is restriction or blockage of blood flow as well as a decreased elasticity of the vascular wall. The condition exists most often in a subclinical form, with detection only with thorough examination at necropsy. As the lesions progress, however, the likelihood of associated clinical disease increases. Clinical conditions include vascular occlusion, rupture, and thrombosis. The significance of these changes depends on the organs affected and the severity of the lesion.

Vascular conditions associated with atherosclerosis include vascular rupture in penguins²⁴ and a vulture.¹¹ The condition in penguins was sometimes associated with prodromal signs of anorexia and decreased responsiveness to keepers on the morning preceding death. Sudden death occurred with no clinical signs in most cases. Cardiac disease identified on necropsy suggests that dyspnea and exercise intolerance may have been present as unidentified clinical conditions.

Atherosclerosis in turkeys and polar penguins has been associated with aortic rupture.^{15,24} In these cases the rupture is likely secondary to the vascular wall degeneration associated with atheromatous changes. In turkeys, aortic rupture may cause mortality rates up to 20% in male bird flocks. These birds demonstrate atherosclerosis, aneurysmal dilation, and rupture. Aortic rupture in polar penguins was also seen in areas without distinct atheromatous degeneration. The similarities in aortic rupture between these species are interesting. Both species typically demonstrate atheromatous plaques. Areas of aortic rupture are more likely abdominal in turkeys and at the heart base in polar penguins.

Atherosclerosis has also been associated with clinical signs in multiple organ systems because of reduced blood flow to critical organs. Most clinical signs are referable to poor blood supply to the brain or muscles

or to secondary cardiopulmonary disease.² Vascular luminal stenosis related to atherosclerosis in multiple vessels in a 16-year-old cockatoo resulted in clinical lethargy, decreased appetite, and falling off the perch. Clinical signs in multiple species include lethargy, disorientation, seizures, fainting, dyspnea, anorexia, regurgitation, and leg lameness to paralysis. Extensive atherosclerosis in the sciatic arteries of wild turkeys¹⁷ and at the origin of the celiac arteries in white Carneau pigeons⁷ suggests that examination of the abdominal aorta and its branches may identify causes for leg lameness previously undiagnosed in cases of significant atherosclerosis.

DIAGNOSIS

Although multiple avian orders exhibit atherosclerosis; some have demonstrated significantly more disease than others. Species of particular concern to the zoo veterinarian include African gray and Amazon parrots, hornbills, ratites, raptors, and polar penguins. Nonspecific conditions or cardiac disease in these birds should be associated with a high index of suspicion for atherosclerosis. In all avian species the condition should be considered for vague neurologic and cardiopulmonary disease, especially in older patients. An appropriate index of suspicion is the first step in proper diagnosis.

Diagnostic options include both direct and indirect modalities. Atherosclerosis by itself is generally subclinical. Unfortunately for many birds, sudden death is often the first clinical indication of this condition. However, as the index of suspicion for this condition increases, clinical investigations should prove of greater value. Research into related conditions (e.g., heart disease) may demonstrate atherosclerosis.

Multiple studies have attempted to correlate atherosclerosis with changes in serum cholesterol and lipoprotein levels. In humans the relationship between elevated serum cholesterol and atherosclerosis is well known. In pigeons, similar serum cholesterol levels were found in atherosclerotic-prone birds and non-prone birds between 3 and 12 weeks of age, despite atheromatous lesions being present in 13 of 33 birds on examination at 12 weeks of age.⁷ However, other studies have contradicted this; Bavelaar² reported correlations between plasma cholesterol levels and severity of atherosclerosis in chickens, quail, and pigeons, suggesting an association of intrinsically high cholesterol with species predisposed to atherosclerosis.

Routine radiography may identify cardiomegaly associated with atherosclerosis and severe vascular

mineralization. However, the complex anatomy of the avian cranial thoracic region makes identification of all but the most severe lesions difficult in most species. Once changes such as vascular mineralization are evident, disease progression is advanced and opportunities for clinical intervention are limited.

Electrocardiograms (ECGs) are becoming increasingly common in avian diagnostics. Normal findings are available for a variety of avian species, including peregrine falcons, psittacines, and gulls.^{16,18,20,21} Normal ECG values have been published for groups of anesthetized and unanesthetized patients. Anesthesia may improve positioning of the patient and decrease muscular trembling. Conversely, some have reported anesthesia-associated arrhythmias.^{4,26} Because atherosclerotic changes often preferentially affect the aorta, causing luminal constriction and impeding flow, left ventricular enlargement with associated ECG changes is a possible clinical finding. ECG changes associated with left ventricular hypertrophy in humans include an increase in the QRS amplitude and prominent septal Q waves.⁵ Cardiac rhythm abnormalities are associated with atherosclerosis in humans.

Comparative studies in birds will become available as more clinicians use ECG diagnostics in these patients. In many reports the ECG electrodes have been attached to birds by alligator clips. In penguin species this has worked well on anesthetized patients because thick feather coats and animal movement have precluded good-quality examinations on awake patients. Using needle electrodes may prove valuable in penguins.

Ultrasound examinations are feasible in a variety of avian species. As in other cardiac evaluations, this diagnostic modality is becoming more useful as more published reports of normal findings become available. Examinations on small species are impeded by the increased impact of air sacs. Larger species, however, may demonstrate the usefulness of ultrasonography. Transesophageal examinations may be particularly effective at visualizing the heart despite the effect of prominent air sacs.

Another diagnostic modality of potential utility is computed tomography (CT) imaging. Although rapid heart rates of birds have previously impeded the use of CT, newer machines are capable of rapid imaging, minimizing the impact of fast heart rates. CT is used in human medicine for identifying mineralized atheromatous lesions and characterizing vascular stenosis. CT may represent an effective clinical alternative to diagnosing birds when thoracic ultrasound is not feasible or air sac interference impedes adequate examination of the great vessels.

Necropsy examination for detection of incidental and clinical atherosclerosis requires a systematic approach. Cardiovascular examinations should include opening cardiac chambers, examining the myocardium and heart valves, and extending incisions to major vessels to examine the intimal surface of vessels at the heart's base. Examination of the aorta should extend from the heart base along the thoracic and abdominal aorta to the level of the celiac arteries. Multiple arteries may be affected, and histologic changes in myocardial, splenic, gastric, and meningeal vessels may be seen only if these organs are examined histologically. Arteriosclerotic and atherosclerotic changes may vary from minor, firm thickenings of the tunica intima to circumferential, hard thickenings of vessels with extensive luminal stenosis. Samples of the affected areas should be collected in 10% neutral buffered formalin and processed routinely for histologic review.

SPECIES AFFECTED

Avian species affected by atherosclerosis vary widely, and multiple reviews for species prevalence have demonstrated the condition across most avian orders. The prevalence of atherosclerosis in each study is likely influenced by the desire of the prosector to detect the atherosclerotic lesions. The incidental nature of these lesions makes a consistent examination across necropsies and examiners uncommon. Thus a variation in prevalence of 2% to 25% likely reflects a difference in examination techniques as well as true differences in prevalence of the lesion.

In a review of pathology cases at the San Diego Zoo from 1960 to 1978 directed by a single pathologist, arteriosclerotic lesions were identified in 11 orders of birds. This overview of pathologic findings from more than 12,000 records serves as a general accounting rather than an in-depth description. Regardless, arteriosclerosis and atherosclerosis were often an incidental finding in these birds. The findings in the Falconiformes are of particular interest, with 14 affected birds from a total of 129 examined. These birds were often of unknown age but had been in the zoo collection for 2 to 45 years. Four of these birds had thyroid enlargement concurrently; three had concurrent myocardial infarcts. One white-backed vulture had a coronary artery rupture and associated cardiac tamponade.¹¹

In a report from the Oklahoma City Zoo, 14 avian orders were identified as affected with spontaneous arterial disease. This review focused on atherosclerosis and identified true atheromatous changes in 24% of 72

birds examined. Of this group, 65 birds (90%) demonstrated some degree of arteriosclerosis. This study described the most advanced atheromatous changes in Galliformes (turkeys and peacocks) and Ciconiiformes (storks and herons).³

A retrospective of submissions to a zoo pathology reference laboratory found an atherosclerosis prevalence of 2.1%, representing 17 of 23 orders of birds examined. Coraciiformes (hornbills, kingfishers, and rollers), Struthioformes (ratites), and Falconiformes (raptors) were most often affected. The condition was considered the primary cause of death in 26% of the birds identified as affected with atherosclerosis.¹⁰

In a review of 57 penguin cases, representing seven species, from one SeaWorld facility over a 5-year period, atherosclerosis was identified as an incidental finding in 21 birds, and 4 additional birds had atherosclerosis as the cause of death. This high prevalence of atherosclerosis may reflect the older population examined or a specific interest of the primary pathologist in demonstrating the lesions. Species affected most often were the Adelie (*Pygoscelis adeliae*) and emperor (*Aptenodytes forsteri*) penguins. The increased prevalence in these species may be related to the much older animals examined compared with the other species.

In reviews of atherosclerosis in psittacines, the condition is found to be pervasive; there was an increased prevalence in African gray and Amazon parrots.² Affected species included a variety of macaws, Amazon parrots, and cockatoos; most cases were associated with sudden death. When noted, clinical signs included dyspnea, lethargy, and neurologic conditions.

Species of particular interest to the laboratory animal community include pigeons, chicken, turkeys, and the Japanese quail.¹⁹ Conditions in these species are well studied and often have species-specific peculiarities. For example, the lesions in pigeons occur primarily at the celiac artery bifurcation of the aorta. The coronary vascular lesions are primarily in the small, intramyocardial arteries rather than the main branches at the heart base.²³

As our knowledge progresses, however, better models for humans are making avian models for atherosclerosis unnecessary. Knowledge of factors and conditions related to atherosclerosis are moderately well understood in birds because of historical work.

ETIOLOGIC CONSIDERATIONS

Atherosclerosis in chickens has been associated with infection from the Marek's disease virus.⁸ These lesions are a fatty proliferation in aortic, coronary,

celiac, gastric, and mesenteric arteries. The suggested etiology is an altered lipid metabolism. No other avian species has demonstrated atherosclerosis with a viral association.

The link between genetics and atherosclerosis likely resides in the structure and regulation of genes for lipoproteins, their receptors, and the enzymes related to their metabolism. In humans, mutations in the low-density lipoprotein (LDL) receptor lead to elevations in cholesterol that are two or three times normal for analogous patients.¹³

Multiple impacts of diet composition are of interest in the etiopathogenesis of avian atherosclerosis. Leghorn chickens fed low-protein diets demonstrated a decrease in the severity of coronary atherosclerosis. Day-old Leghorn chicks were placed on protein-restricted diets. Over 20 months, these birds were sacrificed and the coronary arteries and aorta examined for atherosclerotic changes. Protein-restricted birds demonstrated elevated plasma cholesterol, but no elevation in hepatic cholesterol concentrations. Aortic atherosclerotic lesions did not demonstrate changes associated with protein restriction.⁹ This study is supported by others showing that these two vascular sites react differently to atherogenic stimuli.

Increasing dietary cholesterol concentrations in atherosclerosis-susceptible Japanese quail is directly related to increased serum total cholesterol and severity of atherosclerosis.¹² Psittacines such as budgerigars have demonstrated hypercholesterolemia and severe atherosclerosis on experimental diets supplemented with 2% cholesterol. Many birds with naturally occurring disease have histories of poor diets. A review of cases from the Philadelphia Zoo demonstrated that changing from a seed-based parrot diet to a complete pelleted diet resulted in a decrease in the severity of atherosclerosis in the great vessels.²

Overall dietary excesses are a common concern for many zoo birds. This could play a role in atherosclerosis for birds on balanced diets with no excess cholesterol intake. Dietary excesses with exercise restriction may promote atherosclerosis in birds with otherwise acceptable diets.

Elevated intake of polyunsaturated fatty acids is known to have beneficial effects in humans. The effect of these fatty acids may be related to antithrombotic properties, endothelial stabilization, and antiinflammatory actions. A study comparing muscle and adipose concentrations of alpha-linolenic acid (ALA) to relative atherosclerosis in parrots concluded that increased intake of this fatty acid may have had a protective effect.² Related studies examining differing

dietary ALA concentrations did not demonstrate changes in plasma cholesterol.

DISEASE MANAGEMENT

Management of avian atherosclerosis requires improved clinical diagnoses. Once these are achieved, either through antemortem or postmortem investigations, medical management may be instituted. For situations involving postmortem diagnosis, management should focus on assessment and care of conspecifics. Species predilections should be considered. Evaluations should include a review of feeding strategies for dietary completeness, fatty acid profiles, caloric density, and overall diet availability. Breeders should consider the genetic basis of atherosclerosis when selecting the fittest pairs for reproduction. Veterinarians should review exercise options for individual animals and attempt to minimize environmental or social stress.

When diagnostic evaluations demonstrate atherosclerosis antemortem, individualized patient care should be paramount. Medical management should focus on addressing secondary conditions, such as cardiac, pulmonary, and central nervous system disease. Conditions such as thrombosis and vascular occlusion should be considered as possibilities. Therapy to reduce dietary nutrient imbalances, increase dietary ALA concentrations, reduce overall weight, and gradually increase exercise should be considered.

Newer therapies in human medicine may soon prove valuable for avian patients. Medications such as cholesterol-lowering drugs (e.g., statins) inhibit cholesterol synthesis in humans. These drugs are particularly effective in humans with a significant genetic component to elevated cholesterol levels but are not used when significant left-sided heart failure is present. Their effects in birds have not been studied.

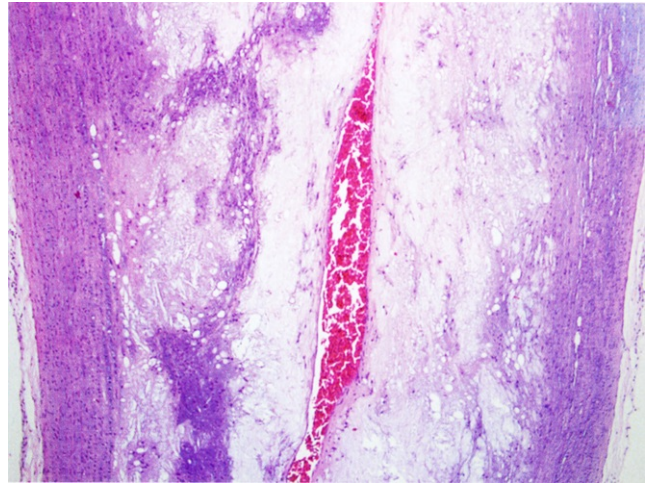
A prophylactic effect was identified using *paracetamol* (crocin) in preventing experimental atherosclerosis in quail. This drug is available over-the-counter in most countries and has antiinflammatory effects. Experimentally demonstrated actions include support of nitric oxide action in endothelial cells in vitro and decreasing levels of oxidized LDPs. These products play an important role in both atheroma initiation and progression.¹⁴

A newer and developing therapy for atherosclerosis involves *plasma delipidation*. Blood is removed from the patient through a catheter, subjected to apheresis for the removal of cholesterol, and transfused back into the patient. This technique may result in rapid regres-

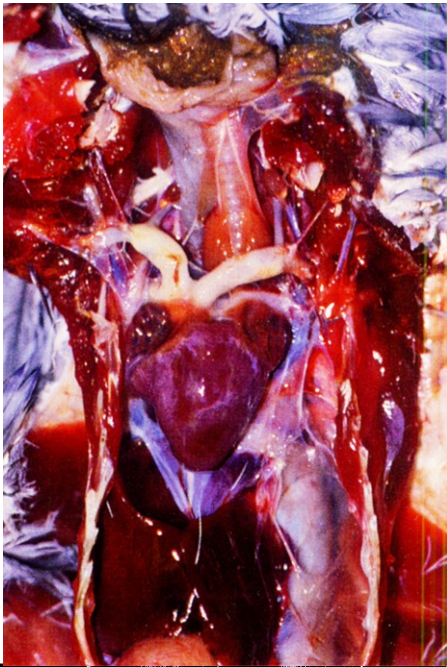
sion of atherosclerotic plaques.⁶ Again, early diagnosis of avian atherosclerosis is the key to effectively using these techniques.

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Color Plate 25-1 Photomicrograph of the aorta from aged Amazon parrot. Note the luminal compromise caused by marked thickening of the wall. (For text mention, see Chapter 25, p. 201.)



Color Plate 25-2 Diffuse and multifocal thickening of vessels at base of the heart in aged Amazon parrot. (For text mention, see Chapter 25, p. 201.)

CHAPTER 26

Minerals and Stork Nutrition

ANDREA L. FIDGETT AND ELLEN S. DIERENFELD

Storks are medium-sized to large waterbirds belonging to the family Ciconiidae, within the avian order Ciconiiformes alongside herons, ibises, and spoonbills. Storks are widely distributed in tropical, subtropical, and temperate wetlands, although many may live and feed where water is scarce. Nineteen species are recognized; all have long bills, necks, and legs; and the largest members of the family are among the largest flying birds. A male marabou (*Leptoptilos crumeniferus*) may be 152 cm (61 inches) tall and weigh 8.9 kg (18.6 lb). By contrast, Abdim's stork (*Ciconia abdimii*) measures just 75 cm (30 inches) and weighs 1.3 kg (2.8 lb).⁶

Some species are gregarious and form large flocks, but most habitually forage alone, especially outside the breeding season. Storks are monogamous and mostly nest in trees. Chicks are nidiculous, initially with little feathering and just a coat of down. They are cared for and fed typically by both parents with food that is regurgitated onto the floor of the nest. Body growth is initially rapid, then slows after approximately 3 weeks as the flight feathers start to emerge; fledging takes place between 50 and 100 days depending on size, with larger species taking longer to mature. Accurate details of reproductive success are limited to a few species but likely range from one young per pair in a year in the larger species, to a maximum of three in the smaller species. The white stork (*Ciconia ciconia*) in Europe, probably the most closely monitored, has an average success of about two young successfully fledged per pair, per year.⁶

Although only seven species are considered under threat, most wild populations are fragmented and struggling.¹⁴ Conservation efforts coordinated through the Storks, Ibis and Spoonbills Specialist Group identified several priorities, including the promotion of captive breeding as a means of building assurance populations.^{16,27,28}

A census conducted by questionnaire in 1987 found all species except Storm's stork (*Ciconia stormii*) were present in zoos, although only four species had world captive populations greater than 100 individuals.²²

The Stork Interest Group was formed to develop programs for some of the rarer species, using experience gained from the breeding success of these more common species. Data current to September 2006, maintained by the International Species Inventory System (ISIS), once again indicated that all but one species, in this case the Asian openbill stork (*Anastomus oscitans*), are present in zoos (Table 26-1). Even accepting that the ISIS data may be incomplete because of the rapidity with which animal inventories may change, that delays may occur in transaction records being logged, or that not all zoos worldwide are members of ISIS, the number of species with world populations greater than 100 individuals is still only seven.¹³

Significant advances in several aspects of husbandry, including enclosure design and holding birds in appropriate social groupings, have increased the number of successes, significantly the Storm's stork at the Zoological Society of San Diego.* Nonetheless, evidence of reproductive difficulties is more compelling given that eight of the species had populations of 25 or less individuals and 10 species had not recorded any breeding activity in the previous 6 months.¹³ Chicks that do hatch are not without problems, as shown by the experience with two lesser adjutant stork (*Leptoptilos javanicus*) chicks hatched at the Wildlife Conservation Society's Bronx Zoo. The chicks were parent-reared and fed diets comprising whole rodents and a supplemented meat mixture containing 2.5% calcium on a dry matter (DM) basis, but both still developed leg bone and beak lesions, which responded favorably to high calcium supplementation.¹

Food availability may be the most important limiting factor in most aspects of wild stork ecology, including distribution, longevity, breeding success, and population numbers.¹⁴ Thus, it is not an unreasonable assumption that nutritional factors may well underlie captive health and successful reproduction in these altricial, rapidly growing species.

* References 3, 8, 10, 23, 29, 30, 41.

Table 26-1

Species and Numbers of Storks (Male.Female.Unknown) in Captivity*

Common/Scientific Name	REGION						Total	% Sp.
	Europe	N. Am.	S. Am.	Asia	Africa	Aus.		
Mycteriini								
American wood stork <i>Mycteria Americana</i>	1.1.1	2.2.3	2.2.3	1.0.0			6.5.7	1
Milky stork (VU) <i>Mycteria cinerea</i>		11.19.4		8.12.49			19.31.53	5
Yellow-billed stork <i>Mycteria ibis</i>	33.24.3	21.20.3		0.0.6			54.44.12	5
Painted stork (NT) <i>Mycteria leucocephala</i>	0.1.0	13.11.9		0.0.5			13.12.14	2
Asian openbill stork <i>Anastomus oscitans</i>								
African openbill stork <i>Anastomus lamelligerus</i>	1.1.0	15.8.0					16.9.0	1
Ciconiini								
Black stork <i>Ciconia nigra</i>	61.42.24	6.7.4		4.4.6			71.53.28	7
Abdim's stork <i>Ciconia abdimii</i>	23.23.8	30.32.34					53.55.42	7
Woolly-necked stork <i>Ciconia episcopus</i>	2.3.0	2.3.0		2.2.11			6.8.11	1
Storm's stork (EN) <i>Ciconia stormii</i>		11.4.0					11.4.0	1
Maguari stork <i>Ciconia maguari</i>	2.6.6	5.5.0	0.0.6				7.11.12	1
White stork <i>Ciconia ciconia</i>	238.218.357	46.32.3		0.2.1	5.5.2		289.257.363	42
Oriental stork (EN) <i>Ciconia boyciana</i>	12.13.0	2.1.0		29.26.14			43.40.14	4
Leptoptilini								
Black-necked stork (NT) <i>Ephippiorhynchus asiaticus</i>		2.1.0		3.5.1		5.2.2	10.8.3	1
Saddlebill stork <i>Ephippiorhynchus senegalensis</i>	13.11.1	30.36.6	1.1.0	3.3.1	3.3.0		50.54.8	5
Jabiru stork <i>Jabiru mycteria</i>			2.3.0					5
Lesser adjutant stork (VU) <i>Leptoptilos javanicus</i>	1.0.0	5.4.4	6.4.0				12.8.4	1
Greater adjutant stork (EN) <i>Leptoptilos dubius</i>	0.0.2	1.0.0		1.0.0			2.0.2	<1
Marabou stork <i>Leptoptilos crumeniferus</i>	108.72.23	57.40.1	0.0.3	1.3.8	7.5.4		173.120.39	15

*As determined by data from International Species Inventory System (ISIS), current to September 2006.¹³ Where appropriate, current conservation status is indicated by IUCN Red List categories: EN, endangered; NT, near-threatened; VU, vulnerable.¹⁴

N. Am., North America; S. Am., South America; Aus., Australia; % Sp., percentage of all species.

FEEDING MORPHOLOGY, STRATEGY, AND DIGESTION^{6,26}

All stork species are exclusively carnivorous, with plant matter ingested only accidentally along with prey (Table 26-2). The bill is comparatively large, with the shape variable from one genus to another according to feeding habits. The four species of *Mycteria* are specialist feeders on aquatic prey, taking mostly small to medium-sized fish (typically 3-30 cm [1.2-12 inches] in length) but also some crustaceans, amphibians, and small reptiles. All have a long, tapered, slightly decurved bill with sensitive parts at the tip that allow the stork to fish in conditions that for many other species would prove impossible. Prey detection and capture are based on touch rather than vision because the species frequent shallow, muddy waters or mudflats. Milky storks (*Mycteria cinerea*) feed largely on mudskippers

(*Periophthalmus*), sometimes by immersing the whole bill and head in mud.

The two species of *Anastomus* share a unique bill with a distinct opening apparent between the two mandibles, giving it the appearance of being deformed. In fact, the bill allows these birds to exploit a particular food item, especially freshwater molluscs and apple snails (*Pila* spp.). Rather than act as a means of cracking snail shells, birds use the tip of their beak to snip the muscle behind the operculum for easy extrication of the snail.

The genus *Ciconia* contains the most familiar storks. All members have medium-sized to large bills, which are probably the least impressive of the family. They are extremely adaptable birds that take many different types of prey in varied habitats, and their bills are thus suited to rather general foraging habits. Abdim's stork may be considered an exception to the "generalist" tag

Table 26-2

Prey Items Recorded in Diets of Free-Ranging Storks

	Adult	Chicks
American wood stork	Fish ²¹	Mainly fish (including killifish <i>Molliensia</i>) ¹⁷
Milky stork	Fish, mudskippers (<i>Periophthalmus</i> spp.) ^{6,21}	
Yellow-billed stork	Fish ²¹	
Painted stork	Fish ²¹	Water snakes ⁴⁶
Asian openbill stork	Freshwater snails (<i>Pila</i> spp.), bivalve molluscs ⁶	
African openbill stork	Freshwater snails (<i>Pila</i> spp.), bivalve molluscs ^{6,12,40}	
Black stork	Fish, frogs ²¹	Fish (particularly <i>Salmo trutta</i>), frogs, reptiles, insects, moles ¹¹
Abdim's stork	Insects (particularly locust swarms, caterpillars of armyworm moth <i>Spodoptera exempta</i>) ⁶	Toads (<i>Bufo</i> spp.), grasshoppers (<i>Oedaleus</i> spp.) ⁷
Woolly-necked stork	Fish, frogs, snakes, lizards, insects, marine invertebrates ^{15,21}	
Storm's stork	Fish, frogs, snakes, lizards, insects ²¹	
Maguari stork	Frogs, fish, reptiles, insects, crustaceans, small mammals, eggs and young of marsh-nesting birds ¹⁸	Eels, fish, earthworms ¹⁸
White stork	Frogs, earthworms, mice, insects, fish ²¹	Insects (particularly Orthoptera, Coleoptera), molluscs, and vertebrates ⁴⁵
Oriental stork	Frogs, earthworms, mice, insects, fish ²¹	
Black-necked stork	Fish (particularly catfish), frogs, coots, ducks ^{31,35}	
Saddlebill stork	Fish, frogs ²⁰	Fish (particularly lungfish <i>Protopterus aethiopicus</i> and catfish <i>Clarias</i> spp.) ²⁰
Jabiru stork	Frogs, fish, snakes, snails, insects ^{18,20}	
Lesser adjutant stork	Fish, frogs ¹⁹	Frogs ¹
Greater adjutant stork	Carrion (including buffalo vertebral column and offal), fish, frogs, reptiles (including flapshell turtle <i>Lissemys punctata</i>), ducks, and bone ^{19,37,42}	
Marabou stork	Carrion, fish, frogs ²¹	

because it is habitually observed in large feeding flocks feasting on swarms of locusts or armyworm caterpillars (*Spodoptera exempta*). In many parts of Africa, together with the European white stork, they are known as “grasshopper birds.” The typical foraging method consists of stalking, by walking slowly while actively looking, then jabbing with bill once prey is sighted.

The genera *Ephippiorhynchus* and *Jabiru* feed mostly on fish of up to at least 500 g and will also feed on other aquatic species, both vertebrate and invertebrate. These species tend to walk rapidly through shallow water to disturb prey, then make jabbing motions to catch items with bills that are very long, pointed, and slightly upturned. However, the largest bills in the Ciconiidae belong to members of the genus *Leptoptilos*, and two species, marabou and greater adjutant storks (*L. dubius*), are notable scavengers regularly feeding on carrion, a feeding preference not shared by any other stork. Although the bills are large (almost 35 cm [14 inches] in the marabou), they are also heavy and ineffective at dismembering carcasses. Nonetheless, they may accommodate large quantities of meat whole (up to 1 kg [2.2 lb]) and are serious predators, consuming a diverse range of live prey, including both young and adult birds. Large bones may also make up a portion of diet (Figure 26-1).

Although absolute nutrient values will vary across prey types, the general similarity in the chemical composition of foods of animal origin permits a relatively similar digestive strategy across subcategories (e.g., piscivores, insectivores). Animal tissues are readily digestible, and all carnivorous birds rely on competent autoenzymatic digestive capacity and a capacity to separate highly digestible soft tissues from relatively indigestible components, such as exoskeletons, fur, feather, and scales. Separation may occur before



Fig 26-1 A bone eaten by free-ranging, greater adjutant stork (*Leptoptilos dubius*). (See Color Plate 26-1.)

ingestion, but also occurs in the gizzard, followed by egestion of the indigestible components back out of the mouth. When these elements are not separated, digestibility is greatly impaired.

CHICK GROWTH AND REARING^{17,23,24}

In nature, the nestling period is one of high mortality from various causes, including predation and failure of the food supply. Any mechanism that shortens this period of helplessness and vulnerability confers distinct evolutionary benefits.

Altricial birds hatch with poor locomotory and sensory ability but with highly developed organs related to metabolism (e.g., alimentary tract, lung, liver, kidney). Consequently, the young altricial bird may ingest and metabolize enormous quantities of food relative to their size and grow rapidly during early life. They will reach their adult size almost five times sooner than is typical for mammals of an equivalent adult size and thus have extra mineral requirements associated with this growth. The calcium density of adult birds and mammals is similar, so growing altricial birds typically need to deposit about five times more calcium each day than an equally sized mammal. They therefore require a greater calcium/energy ratio than other taxa, and when calcium concentration is too low, severe metabolic bone disease may develop in just a few days. Development of the large feathers of the body (e.g., primaries) will also contribute to the decrease in growth rates observed in other body parts as the feathers “compete” for incoming nutrients.

If diet modifications are made during chick rearing, it is to feed different types or sizes of animal prey, or food that has been partially digested before feeding. Chicks feed themselves from food regurgitated onto the nest floor by their parents. Table 26-2 summarizes prey items observed being fed to wild stork chicks; the relative proportion of these items often varies over the course of the rearing period; in several examples, aquatic prey (e.g., frogs, fish, invertebrates) and insects predominate early in rearing. Furred or feathered items tend to appear later, presumably when the chick is more competent at producing pelleted egesta. Thus, whole prey should be skinned for feeding during chick-rearing periods to minimize potential problems with gastrointestinal blockages.

NUTRIENT CONTENT OF FOODS

The variability in dietary items may also result in a wide range of nutrient values consumed by storks

Table 26-3

Select Nutrient Recommendations for Storks* and Published Composition Values for Prey Items Similar to or Present in Diets of Free-Ranging and Captive Birds^{2,4,43,47}

	AS %DM				
	%DM	CP	Fat	Ca	P
Recommendations		24-38	9	0.40-1.00	0.40-0.80
Earthworm <i>Lumbricus terrestris</i>	25	32	13	0.97	0.79
Locust [†] <i>Locusta migratoria</i>	59	52	32	0.04	0.43
Blue mussel <i>Mytilus edulis</i>	19	61	11	0.13	1.01
Crayfish [‡] <i>Orcronectes</i> spp.	31	46	3	14.6	1.0
Eel <i>Anguilla</i> sp.	31	58	37	0.06	0.68
Channel catfish <i>Ictalurus punctatus</i>	20	83	14	0.07	1.06
Smelt <i>Osmerus</i> sp.	21	89	10	—	—
Rainbow trout <i>Salmo gairdneri</i>	27	64	23	2.20	1.29
Green frog <i>Rana clamitans</i>	22	71	10	4.29	1.87
Southern toad <i>Bufo terrestris</i>	28	61	14	2.94	1.79
Anolis lizard <i>Anolis carolinensis</i>	29	76	—	5.54	2.88
Mallard duck <i>Anas platyrhynchos</i>	33	63	26	—	—
Day-old chicks <i>Gallus gallus</i>	26	65	22	1.69	1.22
Mouse (domestic) <i>Mus domesticus</i>	32	57	23	2.64	1.91

*Recommendations are derived from National Research Council (NRC) requirements of domestic felids, mustelids, and poultry.³²⁻³⁴
DM, Dry matter; CP, crude protein; Ca, calcium; P, phosphorus.

[†]Fidgett et al, unpublished data.

[‡]Dierenfeld et al, unpublished data.

eating whole prey. Table 26-3 summarizes select nutrient content (dry matter, protein, fat, calcium, and phosphorus) of various prey. Of note, the calcium content of many prey items eaten by wild storks may be quite high.

NUTRIENT RECOMMENDATIONS

Although specific studies of digestive physiology have not been reported with storks, presumably they share

some of the unique enzyme systems of other obligate carnivores (felids, mustelids) as has been shown for other birds of prey; thus storks utilize fats and proteins, rather than carbohydrates, as primary energy sources.²⁵ Therefore, nutrient requirements should be extrapolated from a combination of avian plus felid models, with crude protein in the diet not less than 24% DM (may be up to 40% during growth) and crude fat not less than 9%.³²⁻³⁴ Meat-based diets are likely not limiting in essential amino acids or fatty acids for storks. Whole vertebrate prey and properly supple-

mented meat-based diets (based on felid models) would likewise supply appropriate vitamin and mineral levels to meet nutritional needs of the Ciconiiformes.

As with other carnivorous species, care should be taken that excessive levels of vitamin A in whole prey do not interfere with possible uptake and utilization of other fat-soluble vitamins.⁴³ Dietary sources of vitamin D (again, whole prey) and access to ultraviolet (UV) light must be provided; this may be particularly true for chicks during periods of rapid growth, which may be sheltered under parents' bodies.

Both ecologically and physiologically, mixed carnivore diets appear suitable for storks, comprising whole vertebrate prey (rodents, rabbits, poultry, fish, amphibians, reptiles), commercially prepared carnivore or felid diets, properly supplemented meat, and occasional invertebrate prey such as crayfish and insects. Although frogs are a diet ingredient in nature, particularly during breeding seasons, they are not sustainably harvested for applied feeding programs, so alternatives that duplicate their nutrient content (e.g., high-calcium natural-skin-casing Carnivore Cuisine sausages, Central Nebraska, North Platte, and additional calcium supplements to typical diets) should be administered during chick growth periods.

Based on the body composition of whole frogs, intake data, and clinical response of chicks, 3.5 g of calcium (as a mixture of calcium carbonate and dicalcium phosphate) was supplied to lesser adjutant stork chicks daily during rapid growth to alleviate deficiency problems, in addition to diets comprising whole rodent prey, fed by the parents.¹ Storm stork chicks were successfully hand-reared using 0.01 g calcium carbonate per 100 g food, 3 parts skinned, chopped mouse to 1 part chopped fish (equal portions of trout and smelt).³⁰ Other facilities report using calcium supplements (e.g., calcium lactate, bonemeal) during chick growth periods, or long-bone and beak deformities result.⁵ Calculated rates and estimated quantities of bone deposition in these rapidly growing chicks support the need for these high levels of calcium supplementation during that phase.

CONCLUSION

Bone disorders resulting from mineral imbalances are rare but not unknown in wild birds.^{9,25,48} Naturally occurring, secondary nutritional hyperparathyroidism has been described in nestling and fledgling cattle egrets (*Bubulcus ibis*). Expansion of the cattle egret into suboptimal habitat, with reduced availability of

calcium-rich prey, is a suggested cause. Approximately 30% of the volume of nestling cattle egret diets consists of vertebrate prey, mostly amphibians. Gut contents from affected chicks contained predominantly grasshoppers and crickets; vertebrate prey items were absent.³⁶

The necessity for calcium supplementation for normal chick development was highlighted in this study, but discrepancies between wild and captive diets are unlikely limited to this single nutrient.

Adult body condition also affects breeding success, and factors affecting avian egg composition and quality are being systematically investigated, although primarily in females. Males also have a vital role to play; studies of avian sperm quality are not as common, but there is solid evidence for the influence of nutrition on the quality of semen in felids, which may also apply to carnivorous birds. Jaguars (*Panthera onca*) were fed a vitamin and mineral supplement based on the National Research Council recommendations for the domestic cat (*Felis catus*). No significant differences were found between collections (made by electroejaculation) for semen volume, concentration, motility, and vigor during the trial period. However, a significant decrease was seen in primary sperm abnormalities during the supplementation period. Abnormalities originate during spermatogenesis and may be the result of genetic, environmental, or nutritional factors. Although further analysis of genetic variability and paternity of these animals is warranted, improvement in seminal characteristics in the animals studied was largely attributed to nutritional supplementation.³⁸

Storks may be large, highly visible, and spectacular birds, but our knowledge of their dietary requirements remains poor, despite food availability suggested as the most important limiting factor in their ecology. Readily available means of recording reliable dietary information in free-ranging birds, such as using the stable isotope signature of their feathers,³⁹ require urgent exploration.

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Color Plate 26-1 A bone eaten by free-ranging, greater adjutant stork (*Leptoptilos dubius*). (For text mention, see Chapter 26, p. 209.)

CHAPTER 27

Veterinary Care of Kiwi

WAYNE BOARDMAN

Endemic to New Zealand, kiwi are small to medium-sized, flightless, nocturnal birds.⁶ They may live in native forests, scrub, or rough tussock and inhabit areas from sea level to subalpine.¹⁷ Adults will live on average 20 years in the wild but may live up to 40 years.³⁴ Kiwi are thought to be an early offshoot from the evolutionary line of the primitive flightless ratites but appear to be more closely related to the emu than the moa.³²

TAXONOMY AND GEOGRAPHIC DISTRIBUTION

Originally, three taxa were described, but recent research has now defined six species of kiwi (Table 27-1).¹⁴

IDENTIFICATION^{16,32}

See Table 27-2.

CONSERVATION STATUS

The range and numbers of kiwi have declined since humans arrived in New Zealand. Forest clearance has reduced available habitat and produced fragmented populations. However, the most important factor is the introduction of predators. Possums damage eggs; mustelids and feral cats kill young chicks; and dogs and ferrets may kill adults. Up to 95% of all kiwi chicks are being killed within the first 2 months of life in some areas.²

All six taxa of kiwi are threatened in the wild in New Zealand.¹⁹ The North Island brown kiwi is classified as “seriously declining,” at a rate of 5.8% per year, mainly from intense predation of young birds in the first 6 months of life.³² The Okarito brown kiwi and the Haast tokoeka are classified as “nationally critical,” and the great spotted kiwi and southern tokoeka are

classified as “gradually declining.” The little spotted kiwi have an increasing population size because of their location on predator-free islands.²⁹

Because of this wild population decline, the *Kiwi Recovery Plan* was launched in 1991 with the aim of “maintaining and, where possible, enhancing the current abundance, distribution and genetic diversity of Kiwi.”^{10,31}

Operation Nest Egg was established to “head start” two kiwi taxa: the North Island brown kiwi and Okarito brown kiwi. Eggs are removed from wild kiwi nests, incubated in artificial incubators, and raised in captivity. Birds are released as subadults, usually weighing in excess of 1 kg (2.2 lb).¹⁴ At this stage they are less likely to be killed by a stoat or a cat, but released birds must still be managed, especially when they may be reached by domestic dogs and ferrets. The first release of captive-reared chicks took place in 1995 and has been highly successful.³² Ten captive institutions have been involved in this successful conservation program.

CAPTIVE STATUS

Kiwi were first held in captivity in London Zoo in 1851, but the first captive breeding did not occur until 1945.¹⁴ Currently, kiwi are more often held in New Zealand institutions, and only 13 zoos hold kiwi outside New Zealand (Table 27-3).¹⁴

ANATOMY AND PHYSIOLOGY

Kiwi have several unusual physical features. All taxa share the following general characteristics: small eyes but good night and day vision; large ears with a good sense of hearing; a well-developed sense of smell, the nostrils being uniquely placed at the tip of the long, sensitive bill; feathers with unlinked barbs on a single rachis that is easily shed; vestigial wings rendering

Table 27-1

Common and Scientific Names and Distribution of Kiwi

Common Name	Scientific Name	Distribution
North Island brown kiwi	<i>Apteryx mantelli</i>	North Island
Okarito brown kiwi or rowi	<i>Apteryx rowi</i>	Okarito district of the South Island
Southern tokoeka	<i>Apteryx australis</i>	Stewart Island
Haast tokoeka	<i>Apteryx australis</i> "Haast"	Haast district of the southern part of the South Island
Great spotted kiwi or roroa	<i>Apteryx haastii</i>	North part of the South Island
Little spotted kiwi	<i>Apteryx owenii</i>	Kapiti Island and several other offshore islands

Table 27-2

Physical and Reproductive Characteristics of Kiwi

Common Name	Length (cm)	Weight (kg)	Identification	Egg Laying	Incubation
North Island brown kiwi	~40	F: 2.8	Grayish brown, streaked	May-Feb	M: 70-80 days
Okarito brown kiwi		M: 2.2	lengthways reddish	June-Jan	M/F: 65-75 days
Southern tokoeka			brown	June-Dec	M/F: 70 days
Haast tokoeka			Long ivory bill	June-Dec	M/F: unknown
Great spotted kiwi	~45	F: 3.3	Light brownish gray	July-Dec	M/F: 65-75 days
		M: 2.4	tinged with chestnut, banded horizontally white		
			Large bill and brown legs		
Little spotted kiwi	~30	F: 1.32	Brownish gray, banded horizontally with white	Sept-Jan	M: 65-70 days
		M: 1.15			

Data from Robertson H: Kiwi Recovery Plan, 1996–2006, Threatened Species Recovery Plan 50, Wellington, NZ, DOC.
F, Females; M, males.

Table 27-3

Captive Populations of Kiwi (February 2003)

Taxa	NEW ZEALAND		NON-NEW ZEALAND	
	No.	Institutions	No.	Institutions
North Island brown kiwi	106	15	39	13
Okarito brown kiwi	3	1	—	—
Great spotted kiwi	4	2	—	—
Little spotted kiwi	2	1	—	—

Data from Threatened Species Occasional Publication 24, Wellington, NZ, 2004, DOC.

them flightless; no external tail; and short, powerful legs with three forward-pointing toes equipped with sharp claws.³² Females tend to be 20% to 30% heavier and larger than males and produce one of the largest eggs in proportion to female body weight (eggs weigh about 15%-20% of female weight). Chicks hatch as miniature adults. Females also have paired functional ovaries that are thought to ovulate alternately, and males a distinct phallus that may be used for sexing. Kiwi also have a remnant of a diaphragm, and their body temperature and metabolic rate are lower than in most birds (Table 27-4).

BEHAVIOR AND REPRODUCTION

Kiwi live in pairs, which is site specific, and they maintain and protect permanent territories. During the day

Table 27-4**Body Temperature, Heart Rate, and Respiratory Rate for 23 Juvenile North Island Brown Kiwi during Physical Restraint**

	Temp (° C)	Heart Rate (beats/min)	Respiratory Rate (breaths/min)
Mean	38.8	165	34
Range	36.6-40.3	70-240	12-60

Data from Jakob-Hoff R, Twentyman C, Buchan B: *Kokako* 7(2):11-12, 2000.

they sleep in burrows, but as twilight appears, they become active and begin probing the soil and leaf litter for food with their long bill, often making snorting noises as they exhale soil. They are usually only active for the first part of the night, approximately 4 to 6 hours. Vocally most active in the first 2 hours of darkness, kiwi have sexually dimorphic calls that may transmit up to 1.5 km (0.9 mile) in ideal conditions.³² Birds tend to be most vocal in winter and spring. Juveniles are usually silent in their first year, and some nonterritorial adult or subadult birds rarely call. Birds on nests do not call.

Kiwi reproductive behavior varies between taxa.¹⁷ During courtship, a pair often remain together for hours, making loud grunts and snuffling sounds. The male and female of a pair often feed separately at night but spend about 20% of days together. Pairs are monogamous, which persists throughout the year and between years. Most eggs are laid between May/June and January, with Okarito brown kiwi laying as late as February.

North Island brown kiwi and little spotted kiwi may lay two eggs 2 to 4 weeks apart, whereas other taxa lay only one egg. The large white eggs (125 × 78 mm; 375-430 g) are laid in either a burrow or a hollow log, or sometimes under dense vegetation. Incubation is generally by the male but may be shared by the female in some taxa, and the incubation period may range from 65 to 80 days.³⁰

The chick hatches fully feathered, stands upright within 6 hours, and walks freely by 1 to 2 days. Chicks have a large internal yolk sac that is gradually absorbed over the first 10 days of life. They remain in the nest for about a week before venturing out unaccompanied. The birds are never fed by the parents. Usually the bird returns to the nest for several weeks but may stay away for the odd day. Chicks seem to stay close to their natal territory for at least 6

to 9 months before dispersing to find a vacant territory. Growth continues for at least 24 months.

ARTIFICIAL REARING

Traditionally, captive-bred eggs have been incubated, but with the advent of Operation Nest Egg, there has been a need to establish standard protocols. The principles of incubation are similar to other species, with a few exceptions. Eggs are dipped in disinfectant (Antec Superhatch Chickguard, NRM, Private Bag 99927, Auckland, NZ) at a temperature of 43° to 44° C for no more than 5 seconds. Eggs are candled, and the air cell is outlined lightly with a soft pencil. The egg is then placed on a clean, flat surface and allowed to find its center of gravity. A pencil line is drawn down the egg's length, indicating top center.

The incubation protocol is split between four age-related stages. Turning is done during days 10 to 55 in a 2-day cycle in 4 × 45-degree stages spaced evenly throughout the day. On day 1 the egg is turned 90 degrees clockwise/to the right in two stages and 90 degrees anticlockwise in two stages. (This will bring the top-center line of the egg back to 12 o'clock on the final turn.) On day 2 the egg is turned 90 degrees anticlockwise/to the left in two stages and 90 degrees clockwise in two stages. This 2-day cycle is repeated as necessary (Table 27-5).

An egg should lose 12% to 16% (0.6-1.2 g/day) of its fresh weight before it internally pips. Hatching takes place in the incubator; the humidity may be increased to 60% when the chick has externally pipped. Wads of cotton may be used to stabilize the egg, which prevents it from moving around. Brooder matting is placed on the bottom of the incubator so that the chick may get traction. Once hatched, the chick is weighed (and thereafter daily for the first 3 weeks) and moved into a Brinsea TLC brooder set at 34° C. After 24 hours the temperature is reduced to 30° to 32° C. After 2 to 3 days the brooder temperature is reduced to 20° to 24° C. After 4 days the chick is moved to a soil-lined brooder with an ambient temperature of 18° to 20° C, where it will stay for 3 weeks. Small stones are added for the chick to ingest, and adult food (cut into smaller pieces) is offered from day 8.

Chicks kept too warm are usually lethargic and slow to eat. It is normal for chicks to lose 25% to 30% of their weight in the first 10 to 12 days. Force feeding should be considered if the weight loss exceeds this percentage.³² After 3 weeks, chicks should have gained their hatch weight and may be moved to an outdoor enclosure.

Table 27-5

Incubation Protocol for North Island Brown Kiwi

Stage	Day	Incubator	Temp (°C)	Humidity (%)	Turning	Cooling
1	0-9	Still-air Brinsea Hatchmaster	36.0-36.5	60-65	None	1 hour/day 8-9 AM
2	10-55	Still-air Brinsea Hatchmaster	36.0-36.5	60-65	4 × 45 degrees daily	1 hour/day 8-9 AM
3	56-internal pip (~75)	Still-air Brinsea Hatchmaster	35.5-36.0	>60	None	None
4	Internal pip– hatch (~75-78)	Still-air Brinsea Polyhatch	35.5	55-60	None	None

Data from Robertson H: Kiwi Recovery Plan, 1996–2006, Threatened Species Recovery Plan 50, Wellington, NZ, DOC.

HOUSING REQUIREMENTS AND ARTIFICIAL INCUBATION/REARING

When possible, all facilities should approximate the wild habitat as closely as possible. Kiwi may be housed singly or in pairs. However, it is important to note that introducing a pair together may lead to major injury or death. During the day, kiwi sleep in a small, rectangular burrow (~1 m × 30 cm × 45 cm), which may be placed on or under the ground, as long as it is well sheltered from excessive heat and rain. More than one burrow should be offered. The roof should slope if outdoors and must have access for caregivers so that the bird may be checked or caught.

A dry, leaf litter substrate provides a cool, dry habitat and should be replaced regularly. Kiwi are usually kept in two types of enclosure: nocturnal display and off display. It is usual practice to exchange display birds so that they may be offered fresh ground off display to exhibit unrestricted normal behaviors. Each enclosure should be well planted to provide cover. Temperature, humidity, and photoperiod should vary seasonally if on display. These areas should have a concrete floor, covered with a deep layer of soil and leaf litter, which is replaced at regular intervals to reduce parasite buildup. Lighting should mimic both the photoperiod and night length so that kiwi may be seen when they are most active, in the first few hours of darkness. The environmental temperature should be controlled to ensure it is between 5° and 20° C and should vary over the day and between seasons. Artificial ultraviolet (UV) lighting should be offered for short periods during the day if on display. Kiwi are likely to return to their burrows after only a few hours and thus be off display; a second display area with a

staggered daylight period may then be used. Ventilation should be adequate. Double-glazed windows are important for exhibits to reduce vibration and noise. Sufficient activities and furniture should be provided to prevent repetitive behaviors; if these occur, birds should be moved to an off-display area.

DIET

Free-Ranging State

In the wild, kiwi locate and feed on worms, insect larvae, weta, crickets, centipedes, moths, earthworms, spiders, and fallen fruits and berries (although they have also been recorded occasionally feeding on leaves) on the surface, from rotten logs, and by probing up to 10 cm (4 inches) in the ground.³²

Captivity

A recent survey in New Zealand indicated a wide range of captive diets, although none of the diets approximated wild kiwi diets, as determined by analysis of the gizzard contents of dead birds, and vegetable matter may be a much more important food source than first thought.¹⁸ The captive diet presently consists of lean ox heart and tofu (soy) cut into julienne strips, sultanas and banana, diced fruit, peas with added yeast and wheatgerm flakes, sunflower oil, vitamin supplement, and calcium carbonate offered once daily at night. Daily provision of rotten logs, fresh leaf litter, and mulch with added earthworms and other invertebrates encourages normal probing activity.

Many captive birds are overweight, and the breeding success may be poor. Wild birds appear to have a seasonal weight pattern, often dramatically increasing their weight at breeding. The lack of seasonal variation in body condition in captive kiwi may account for smaller eggs and chicks bred in captivity. Weighing birds regularly is thus important, to offer food in order to reduce the weight in the nonbreeding season. Allowing for an increasing plane of nutrition before the breeding season may be important.

HANDLING, SIGNS OF POOR HEALTH, AND IDENTIFICATION

Kiwi should be handled carefully because they are easily injured; they have a minimal sternum, weak pectoral muscles and ribcage, and a long, thin bill. They also have an ability to shed feathers easily. In addition, kiwi are able to seriously injure handlers with their claws. A firm grip around the bare part of both legs, with the body cradled on a forearm with the bird's head tucked into the holder's axillary region, is usually sufficient to prevent injury to bird and handler. Birds should be transported in padded boxes; avoid padding that may come loose and be ingested.

Kiwi mask signs of illness well. Presenting signs may include dry, "spiky" plumage, sitting in water dishes,²¹ inappetence, weight loss, diarrhea, inability to stand, pyrexia, and heart murmurs. Birds may be found lying on their side, kicking repeatedly, or may show signs of imbalance. "Tripoding," in which the bird uses both legs and its bill to maintain an upright stance, is an indication of generalized weakness. Daylight feeding may often be an indication of a nonspecific health problem.

On examination, an individual bird is said to be in "poor condition" when the ribs feel like a "washboard" and the vertebral column is clearly felt. If neither the vertebral column nor the ribs may be felt, the bird is in "good condition."³²

Large, strong, steel leg bands are the best method of identifying kiwi. Transponders may also be used in juvenile kiwi. The site usually recommended is subcutaneously over the right thorax caudal and ventral to the vestigial wing.³²

ANESTHESIA

Isoflurane inhalation anesthesia using a mask has been used to good effect in kiwi employing standard avian techniques. The procedure is simplified because of

the distally placed nostrils, which means only a small mask is needed for induction. However, struggling kiwi need to be held firmly so that the bill is not injured. Intubation is easily achieved using 2 to 4-mm endotracheal tubes. Care must be taken when anesthetizing gravid birds because of the large size of the egg.

DIAGNOSTIC SAMPLING

The only accessible venipuncture site is the median metatarsal vein. Blood sampling is easier to perform when the bird is anesthetized, although the conscious bird can be restrained on its back with the legs pointed toward the sampler. A 1-mL syringe with a 25- to 26-gauge needle may be used. Preheparinized syringes may minimize clotting.

Fecal checks should be performed on all adults every 4 months. Juveniles should be checked more regularly, particularly if coccidiosis has been seen previously.

All sick kiwi should be radiographed because foreign body ingestion and peritonitis are common occurrences.

DISEASES

Noninfectious Diseases

The most serious causes of trauma and death are related to predators, which include dogs, cats, and introduced stoats. The main concerns if the kiwi survives are severe cloacal damage and ensuing cellulitis. Aggressive exchanges between captive birds often result in the death or injury of one of the birds. Trauma to the bill tip has been seen in captive kiwi. Repair is difficult because of the narrow, fragile nature of the bill. Despite attempts at repair, the bill tip may often become ischemic, although some birds have been known to adapt.

Some birds, caught in illegal gin-traps, may be brought to a captive facility. Often the leg is irreparable, and amputation is indicated. Birds may often adapt if the amputation is low on the leg, but amputees may only survive for short periods.

Embryonic mortalities are often seen in kiwi eggs that have been incubated artificially. However, improvements in incubation techniques have reduced the number of problems encountered. Bacterial contamination and abnormalities in yolk sac internalization are typically seen in eggs subjected to high incubation

temperatures. Chick and embryo deformities have been seen, including crossed or bent bills, curled toes, and anophthalmia. Angular limb deformity has also been seen in hatchlings.

One of the common problems encountered in the first 3 weeks of life is retention of the yolk sac. Normally, the yolk should be absorbed within the first 2 weeks, but in certain cases, yolk digestion and absorption slow or cease. Clinical signs include continued weight loss beyond the normal weight loss in the first 10 days, failure to eat, weakness, depression, dyspnea, abdominal distention, and often the inability to stand correctly. The retained yolk sac is often 20% to 40% of the chick's body weight. Diagnosis is based on the symptoms, a doughy mass on abdominal palpation, and radiography, which reveals an enlarged mass. The etiology is not clearly understood but includes suboptimal incubation conditions, excessive handling, systemic disease, or infection of the sac.

Ingestion of foreign bodies has been a common cause of death in adult kiwi. Pieces of metal and soft materials may be found in the substrate and are readily ingested, leading to perforation of the stomach wall or blockage. Surgical removal and treatment with broad-spectrum antibiotics have been successful. It is important when changing the substrate to check for foreign bodies using a metal detector.

Egg peritonitis is a common cause of mortality in breeding females in captivity^{8,16} but has also been seen in wild kiwi.³⁰ It has also been associated with hepatic hemorrhage associated with a fatty liver.¹⁶ Egg binding also occurs and may be treated surgically by a hysterotomy.¹² It is important to examine breeding females gently, especially immediately before egg laying, and to ensure the birds are not overweight.¹⁶

Visceral gout has been seen on several occasions in chicks, which are usually found dead. Visceral gout was associated with congenital ureteral obstruction in one neonate.

Several kiwi in a group developed lesions resembling seborrheic dermatitis. Exudative encrustations were seen on the head, around the mouth and ears, and later, on the feet.⁸ Histologically, there was a marked hyperkeratosis with exfoliating sheets and inflammatory crusts associated with mixed bacteria and microabscesses.⁸ The history indicated that the vitamin supplementation had been absent from the diet for only 3 weeks. Treatment with B vitamins quickly and successfully reversed the signs. Similar conditions have been reported at other captive institutes. The condition most closely resembles biotin or pantothenic acid deficiency, which is seen in domestic chickens.⁵

Degenerative eye conditions have been seen during a survey of wild Okarito brown kiwi.²⁷ Ocular abnormalities were seen in 36% of the population and included buphthalmia (1 eye affected), phthisis bulbi (2 eyes affected), corneal edema (4), corneal vascularization (2), nuclear sclerosis (8), cataracts (1), subluxated and luxated cataractous lenses (3), and vitreal opacity (1). Three adult kiwi in good condition had chronic ocular lesions associated with severe visual dysfunction. The nature and frequency of ocular lesions suggest this population is older.

Generalized steatitis, hepatic lipidosis, hemosiderosis, atherosclerosis, and goiter have also been seen infrequently.

Pneumoconiosis is often seen as an incidental finding in kiwi.³³ It is thought to be associated with the inhalation of fine dust particles through the distally placed nostrils.

Tetramisole toxicity and death have occurred when birds were dewormed with the anthelmintic Aviverm. The dose rate was not reported, so it is not known whether this is a species-related or a dose-related sensitivity to the drug.

Infectious Diseases

Antibiotic-responsive vestibular disease has been seen in young kiwi associated with ataxia, an inability to stand, central blindness, and pyrexia.²⁰ A marked heterophilia may be seen. Prompt treatment with broad-spectrum antibiotics and supportive care may reverse the clinical signs quickly; in some cases, prolonged supportive care may be needed.

Septicemia associated with a variety of bacteria has been seen in many kiwi, especially juveniles. Pure growths of *Salmonella typhimurium*, *Proteus mirabilis*, and *Escherichia coli* have been isolated from various organs. Pneumonia and septicemia associated with a pure growth of *Pasteurella multocida* have also been seen.

Cryptococcosis has been seen twice in *Apteryx australis mantelli* in New Zealand^{1,2} associated with liver and lung lesions. The organism cultured from these cases was *Cryptococcus neoformans* var. *gattii*. Kiwi have a much lower body temperature (~37.5°C); this may make them more susceptible to cryptococcal organisms, which proliferate in temperatures less than 40°C. This organism is generally associated with two species of Australian gum trees, *Eucalyptus camuldalensis* and *E. tereticornis*.¹⁵ Neither of these two species was confirmed in the kiwi enclosure substrate. It is believed that this species of cryptococcus may be associated with other species of gum tree.²⁹

Aspergillosis has been the cause of death on several occasions in adult kiwi. Birds usually show weight loss while still maintaining a reasonable appetite. Terminally, they show dyspnea and marked depression. Characteristic fungal plaques are seen on air sacs, and granulomata are seen in the lungs and throughout the coelomic cavity.

Generalized avian tuberculosis has been seen in an adult kiwi. Miliary white nodules were seen throughout the liver, on the serosal surface of the spleen, gizzard, and the intestinal tract.

Parasitic Diseases

Coccidiosis has been seen in many kiwi, both wild and captive. The disease is seen in the first 2 years of life, but most often in the first 6 months, and in captive birds is associated with diarrhea, severe dysentery and melena, inappetence, dehydration, and weakness. Birds soon die if not treated. Coccidial organisms have been seen in the large intestines,³ in the renal collecting ductules and pelvis, in the liver parenchyma and bile ductules, and in the pancreas.³⁵ The etiology is an *Eimeria* sp., which is almost certainly species specific. The condition may be treatable with toltrazuril (Baycox, Bayer Ltd., New Zealand) at 10 mg/kg once daily for 2 days. Single doses of 25 mg/kg repeated in 3 weeks have also been used successfully. The area where kiwi live should be rested wherever possible. Removal of substrate and replacement with fresh leaf litter should be considered at least yearly. Regular fecal flotation tests should be performed in juveniles (e.g., every 3-4 weeks until 18 months of age).

Babesia-like hemoparasites, recently named *Babesia kiwiensis* sp. nov., were found on the blood screen of 10- to 20-day-old chicks brought in from the Northland district of New Zealand.²⁸ Clinical signs and clinical pathology thought to be attributed to the parasite included regenerative anemia with reticulocytosis, pyrexia, heart murmur, lymphocytosis, and transient basophilia.²⁴ A further hemoparasite, described as *Hepatizon kiwi* sp. nov., was seen in another bird. Treatment with chloroquine and primaquine and doxycycline failed to reduce the parasite burden. Additional signs of dry, "spiky" plumage with areas of feather loss on the head and body and crusty indurations in the skin at the commissure of the mouth and the margins of the eyelids were consistent with biotin deficiency.²⁴

An untyped plasmodium was seen in a high proportion of young red blood cells in a 3-month-old juvenile with signs of pyrexia, heart murmur, inappetence,

and lethargy.²¹ Although a 4-day course of primaquine and chloroquine was successful in treating the disease, the parasite was not entirely eliminated.²¹

Visceral larva migrans has been seen in several kiwi. Granulomata have been seen in the cerebellum, brainstem, liver, gizzard, and myocardium.⁴ The parasite has not been identified in these cases. In other cases, however, granulomata in the liver, associated with migrating larvae, have been identified as *Toxocara cati* (presumably acquired from feral and domestic cats) and *Capillaria*.¹¹

The spirurid nematodes *Cyrtus apterycis* are found in the gizzard and *Heterakis gracilicauda* in the large intestine.²⁵ Normally these nematodes cause little pathology, except when found in high numbers. Fenbendazole at 25 mg/kg once daily for 3 days may be used,¹² and ivermectins at 0.2 mg/kg once only. Trematodes and cestodes have also been found in adult kiwi. Praziquantel at 10 to 12 mg/kg orally once has been used to treat cestodes.

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CHAPTER 28

Paramyxoviruses in Bats

ANDREW C. BREED

Bats are an extraordinary group of mammals; not only are they the only mammals capable of sustained flight, but they are also found in almost all habitats, having a worldwide distribution, except for the highest mountains and extreme polar regions. Bats also occupy a diverse array of ecologic niches and contribute significantly to mammalian diversity, with more than 1000 species.³⁹

Bats are known to host six paramyxoviruses: Nipah virus, Hendra virus, Menangle virus, Tioman virus, bat parainfluenza virus, and Mapuera virus. At least three of these are able to infect humans and domestic animals. Ever-increasing human encroachment on natural habitats, combined with the ability of some bats to adapt to anthropogenic environmental changes, has led to increased contact between bats and domestic animals and humans. This may be a key reason for the repeated emergence of several paramyxoviruses from bats in recent years. These viruses have been able to jump the species barrier, and in the case of Nipah virus in Bangladesh, then spread from person to person.

DESCRIPTION OF PARAMYXOVIRUSES

There are two subfamilies and six genera within the family Paramyxoviridae. The genera *Morbillivirus*, *Respirovirus*, *Rubulavirus*, and *Henipavirus* make up the subfamily Paramyxovirinae, and the genera *Pneumovirus* and *Metapneumovirus* constitute the subfamily Pneumovirinae.⁵⁶ Each of the six genera contains highly contagious human and animal pathogens.⁶²

Paramyxoviruses have been found predominantly in mammals and birds, and most have a narrow host range in nature, but display a broad host cell range in culture.²⁶ Transmission is generally horizontal, mainly through airborne routes, and no vectors are known.²⁶ Primary replication is usually in the respiratory tract. Infection is generally cytolytic, but persistent infections often occur.⁵⁶

Paramyxoviruses are pleomorphic and 150 to 300 nm in diameter.¹⁸ Virions are made up of a lipoprotein envelope and a nucleocapsid that surrounds a single strand of linear, negative-sense ribonucleic acid (RNA).¹³ Virion proteins common to all genera include three nucleocapsid-associated proteins: a nucleocapsid protein (N or NP), a phosphoprotein (P), and a large putative polymerase protein (L); and three membrane-associated proteins: an unglycosylated envelope protein (M) and two glycosylated envelope proteins, comprising a fusion protein (F) and an attachment protein (G or H or HN).⁵⁶ The attachment and fusion proteins are of primary importance in inducing virus-neutralizing antibodies and immunity against reinfection.^{56,57} Antibodies to other viral proteins are also produced and some, nucleocapsid proteins in particular, are known to play a role as antigens for cytotoxic T cells.³⁷

BATS AS VIRAL HOSTS

Historically, a wide range of viral infections, including flaviviruses, alphaviruses, rhabdoviruses, arenaviruses, reoviruses, and paramyxoviruses, have been identified in bats.⁶⁰ More recently, a number of emerging zoonotic viruses have been detected in bats.³² These include Hantaan virus, isolated from the common serotine bat (*Eptesicus serotinus*) and the horseshoe bat (*Rhinolophus ferrumequinum*) in Korea; Rift Valley fever virus, isolated from the bats *Micropteropus pusillus* and *Hipposideros albae* in the Republic of Guinea; a strain of yellow fever isolated from an *Epomophorus* Old World fruit bat in Ethiopia; and serologic evidence of Venezuelan equine encephalitis, St. Louis encephalitis, and eastern equine encephalitis viruses in bats in Guatemala.³² Although bat-variant rabies has long been recognized in the United States, the prevalence of human rabies cases attributed to that variant has increased in recent years.⁴⁶ Most recently, strong evidence shows that horseshoe bats (*Rhinolophus* spp.) may be the source of the severe acute respiratory syndrome (SARS) coronavirus.^{40,42}



Fig 28-1 Administration of oral rehydration solution to greater flying fox (*Pteropus neohibernicus*) after general anesthesia for application of satellite collar in Papua New Guinea. (See Color Plate 28-1.) (Courtesy Andrew C. Breed.)

Also, Old World fruit bats of the genera *Hypsignathus*, *Epomops*, and *Myonycteris* may be natural hosts of Ebola virus, as found in Gabon and Republic of Congo.⁴¹

Bats may travel hundreds of kilometers (or miles) in a matter of days. Besides having significant implications for disease spread, this also suggests that populations of pathogens carried by bats are likely to be relatively homogenous across wide geographic areas.⁹ Studies of Old World fruit bats using satellite telemetry have shown that individuals can travel more than 2000 km in 1 year and traverse significant bodies of open sea, such as the Torres Strait between Australia and New Guinea and the Strait of Malacca between peninsular Malaysia and Sumatra (www.henipavirus.com)^{10,61} (Figure 28-1). These long-distance movements may transmit pathogens over great distances and enable exchange between bat populations on different land masses.

Population size and density are positively associated with the diversity of pathogens hosted by mammalian species.² A large population size, as seen for many colonial bats, supports pathogen reproduction by providing a constant supply of individuals susceptible to infection and thus allows persistence of the pathogen.³

Regular, but not constant, contact between individual bats from different subpopulations allows for partial connectivity between colonies of bats. A metapopulation may exist where a spatial mosaic involves a constellation of subpopulations of which, at any given time, some are susceptible, some infected, and some immune to a particular disease.⁴³ This is beneficial for genetic diversity and may permit pathogens, particu-

larly viruses, to persist in a species with a total population that would otherwise be too small to maintain the disease.⁸ This results in these species having considerable potential to act as vectors for, and disseminators of, viruses and other pathogens.

Some authors have proposed that bats are unique in their response to viral infection and are able to sustain viral infections without disease.⁶⁰ However, many other small mammals act as reservoirs for viruses without evidence of disease, and recent analysis of a database on all emerging infectious diseases of humans suggests that bats (which represent as much as a quarter of all mammalian species) do not harbor a disproportionate number of the known emerging zoonotic viruses.⁷¹

PARAMYXOVIRUSES OF CHIROPTERA

Hendra Virus

In September 1994, an outbreak of severe respiratory disease of horses occurred in the Brisbane suburb of Hendra in eastern Australia (Queensland)⁴⁸ (Figure 28-2). The index case was a pregnant Thoroughbred mare, and 16 other horses at two sites showed signs of loss of appetite, dyspnea, and copious frothy nasal discharge. Twelve of the affected horses died a few days after the onset of signs.⁷ Two people who had close contact with the index case also became infected. One of them, a stable worker, developed flulike signs and recovered. The other person, a horse trainer, showed

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Fig 28-2 Timeline indicating emergence of henipaviruses. (Eaton BT, Broder CC, Middleton D, Wang L: *Hendra and Nipah viruses: different and dangerous*, Nat Rev Microbiol 4:23-35, 2006, Macmillan Magazines.)

rapid development of respiratory illness and died 11 days later.⁵⁸

It was thought that the pattern of disease in the horses reflected a point source of infection, and that all other cases were a direct result of transmission from the pregnant mare.⁷ A range of pathogens and toxins were investigated and excluded from the diagnosis. A novel virus was cultured from the lungs of five of the affected horses and from the kidneys of the fatal human case.⁴⁹ The virus showed characteristics suggesting it belonged to the family Paramyxoviridae, although there was minimal cross-reactivity between the virus and a range of antisera to other paramyxoviruses. The virus showed 50% homology of the partial M protein gene sequence of several morbilliviruses and thus was initially called equine morbillivirus (EMV).⁴⁸ The name was subsequently changed to Hendra virus (HeV) when it became apparent that horses were not the natural host for the virus and that it did not belong in the genus *Morbillivirus*.

Surveillance of wildlife species identified flying foxes (genus *Pteropus*, family Pteropodidae) as the likely natural host of the virus. The infection was found to be widespread in four of the flying fox species found on mainland Australia: the black flying fox (*Pteropus alecto*), gray-headed flying fox (*P. poliocephalus*), little red flying fox (*P. scapulatus*), and spec-

tacted flying fox (*P. conspicillatus*).^{22,29} Sampling of 46 species of ground-dwelling mammals revealed no evidence of HeV exposure.⁶⁹ Studies of seroprevalence of HeV antibodies in flying foxes in Australia indicate a prevalence of approximately 50%²⁵ (Figures 28-3 through 28-5).

Since the first outbreak, further outbreaks of HeV have occurred in Queensland, including Mackay in 1994, involving fatal infections of both horses and a human; Cairns in 1999, involving a single horse; and Cairns and Townsville in 2004, involving both horses and a veterinarian.²³

Hendra virus infection of terrestrial mammals, including humans, results in a systemic vasculitis with significant pathology of the lung and central nervous system (CNS).^{34,66,70} Viral antigen is detected in vascular endothelium and frequently recovered from nasopharyngeal swabs, urine, and internal organs, including lung and brain.^{19,34} Experimental HeV infection of flying foxes, however, appears to cause only a sporadic subclinical vasculitis, even at infective doses lethal to horses.^{68,69} Viral antigen is detected in the tunica media rather than endothelial cells, which may help explain why flying foxes appear to be spared from clinical disease.²⁰ Experimental infection of flying foxes has also shown placental transfer of the virus to a fetus.³⁴



Fig 28-3 Anesthesia of wild-caught, spectacled flying fox (*Pteropus conspicillatus*), using isoflurane and oxygen, for Hendra virus surveillance in North Queensland, Australia. (See Color Plate 28-3.) (Courtesy Jack Shield.)



Fig 28-4 Collection of oral swab from anesthetized spectacled flying fox (*Pteropus conspicillatus*) for Hendra virus antigen detection. (See Color Plate 28-4.) (Courtesy Jack Shield.)

Nipah Virus

Nipah virus (NiV) was first described in March 1999 in the investigation of an outbreak of disease in pigs and humans in Malaysia (see Figure 28-2). In the course of the outbreak, 265 humans were infected, 105 fatally.¹⁵ Infected pigs were identified as the primary source of human infection, and over 1 million pigs were culled

to control the outbreak. Wildlife surveillance identified the Malayan flying fox (*Pteropus vampyrus*) and island flying fox (*Pteropus hypomelanus*) as probable natural hosts of NiV.³⁸

Subsequent studies have also found serologic evidence of NiV infection in the Malayan flying fox, island flying fox, and Lyle's flying fox (*Pteropus lylei*) in Thailand, in Lyle's flying fox in Cambodia, and in the Indian flying fox (*Pteropus giganteus*) in Bangladesh.^{35,51,63} NiV has strong serologic and sequence similarities to HeV and is the second member of the genus *Henipavirus*.⁶⁴

Subsequent to NiV's emergence in Malaysia, five outbreaks of NiV-associated disease in humans were described in Bangladesh between April 2001 and February 2005.^{4-6,35} As of 11 February 2005, a total of 122 cases had been recognized by the Bangladesh Directorate of Health Services, at least 78 (64%) of which were fatal. A number of the characteristics of the Bangladesh outbreaks were similar to the outbreak in Malaysia: delayed recognition, a primary presentation of humans with fever and CNS signs, and a high case-fatality rate. In marked contrast to the Malaysian outbreak, however, infection in humans was not associated with disease in pigs, and evidence indicated horizontal human transmission. Further, the pattern of the Bangladesh outbreaks suggests a sporadic, geographically scattered introduction of infection to humans. Nucleotide sequence data also support a different epidemiology in Bangladesh. Data obtained from human cases in Malaysia suggest a single source of human



Fig 28-5 Collection of piece of wing membrane from anesthetized spectacled flying fox (*Pteropus conspicillatus*) for molecular genetic studies. This technique is used to elucidate population structure of flying fox species for henipavirus epidemiologic studies. (See Color Plate 28-5.) (Courtesy Jack Shield.)

infection from the porcine amplifying host.^{1,11,15} Data from Bangladesh cases formed a cluster clearly distinct from the Malaysian sequences, but differed from each other by approximately 0.8%, suggesting possibly multiple introductions of virus into humans.³⁰

The pathologic effects of NiV in terrestrial mammals are similar to those of HeV, with infection resulting in a systemic vasculitis and significant pathology of the lung and CNS.^{34,66} In contrast to HeV, however, viral antigen is often found in bronchial and alveolar epithelium. NiV has not been associated with clinical disease in flying foxes.²⁰

Menangle Virus

In August 1997, Menangle virus was isolated from stillborn piglets at a swine farm in Menangle, New South Wales, Australia.⁵⁴ Many of the piglets had craniofacial and spinal deformities and degeneration of the brain and spinal cord. Additionally, the pig herd experienced a reduced pregnancy rate, increased abortion rate, decreased litter sizes, and increased proportion of stillborn and mummified piglets. Infection of humans also occurred; two swine farm workers developed an influenza-like illness and high-titer antibody responses to Menangle virus.¹²

Menangle virus was classified as a member of the Paramyxoviridae based on electron microscopy of the virus grown in cell culture.⁶⁷ Data on nucleotide and deduced amino acid sequences from the viral genome

showed the closest relationship to members of the *Rubulavirus* genus, including mumps and simian parainfluenza type 5.⁶⁷ These preliminary genome sequence data suggested that Menangle virus was a new member of the genus *Rubulavirus* within the family Paramyxoviridae.

A colony of *Pteropus poliocephalus* was known to roost near the affected piggery in Menangle. Antibodies to Menangle virus were found in flying foxes from the colony,⁵⁴ and electron microscopy (EM) revealed virus-like particles in the feces of flying foxes from a nearby colony.⁵⁵

Tioman Virus

During the search for the natural host for NiV, a novel paramyxovirus was isolated from a number of pooled urine samples of island flying foxes (*P. hypomelanus*) from Tioman Island off the eastern coast of the Malay peninsula.¹⁷ Electron microscopy of virus-infected cells revealed spherical, enveloped virus particles compatible in structure with viruses of the family Paramyxoviridae.¹⁶ The virus showed serologic reaction to antibodies to Menangle virus, but not to a number of other paramyxoviruses investigated.¹⁶ Molecular characterization of the nucleocapsid (N) protein gene of the new virus and Menangle virus showed them to be approximately 70% homologous at the nucleotide level and approximately 85% homologous at the amino acid level.⁴⁴ Analysis of the full-length genome

indicated the virus to be a member of the genus *Rubulavirus* within the family Paramyxoviridae, and it was named Tioman virus.¹⁷

The potential for Tioman virus to cause disease in humans, flying foxes, or other animals is unknown.⁴⁴

Bat Parainfluenza Virus

The first recorded isolation of a paramyxovirus from a bat was described in 1971 by Pavri et al.⁵³ The virus was isolated from a suspension of pooled organs from an Old World fruit bat, *Rousettus leschenaulti* (family Pteropodidae), captured as part of ongoing investigations into rabies outbreaks in the district near Poona, India. Hemagglutination inhibition, complement fixation, neutralization tests, and growth characteristics revealed that this virus represented a new parainfluenza strain that was related to but distinct from simian virus 41 (SV41), placing it in the parainfluenza type 2 group.³³ Serosurveys revealed specific neutralization of the bat virus by serum specimens from 7% of 70 *R. leschenaulti* samples tested. Bat parainfluenza antibodies were also demonstrated in 10% of 200 human serum samples tested.⁵³ It is not known whether the observed antibody reactions in humans were caused by interspecies transmission of the virus or a serologic cross-reaction.⁵³

Mapuera Virus

Mapuera virus was isolated from a little yellow-shouldered bat (*Sturnira lilium*), a New World leaf-nosed bat (family Phyllostomidae), from Brazil in 1979. It was tentatively classified as a member of the family Paramyxoviridae on the basis of its morphology and its ability to hemagglutinate guinea pig erythrocytes.⁷²

The molecular biology of Mapuera virus has been studied at both the protein and the nucleic acid levels.³¹ Seven virus-encoded proteins were detected in infected Vero cells. Based on the similarity of N-protein sequences, results indicate that Mapuera virus should be placed within the genus *Rubulavirus*, which includes mumps virus, simian virus 5 (SV5), and Menangle virus.⁵⁶

DIAGNOSTIC TESTS

To date, diagnostic test development has been most successful for Hendra and Nipah viruses. Four diagnostic tests—virus isolation, EM, immunohisto-

chemistry, and polymerase chain reaction (PCR) and sequencing—have been described for the detection of virus or viral antigen of these two viruses. Two diagnostic tests for the detection of antiviral antibodies are serum neutralization (SN) and enzyme-linked immunosorbent assay (ELISA).¹⁹ Because Hendra and Nipah viruses are classified internationally as biosecurity (biosafety) level 4 (BSL4) agents, tests necessarily involving live virus (i.e., virus isolation and SN tests) should only be carried out under physical containment level 4 (PC4) conditions.

Virus Isolation

Hendra and Nipah viruses grow well in Vero cells from a range of tissue specimens, including brain, lung, kidney, and spleen.¹⁹ Cytopathic effect usually develops within 3 days, and virus isolates may be specifically identified by immunostaining, neutralization with specific antiserum, PCR, and EM.

Immunohistochemistry

Immunohistochemistry (IHC) may detect viral antigen in a range of tissues. Because IHC uses formalin-fixed tissues, the technique is useful for retrospective investigations on archived materials, and the biosafety constraints of viral isolation and SN tests do not apply. The availability of a range of polyclonal and monoclonal antisera allows that test sensitivity and specificity to be tailored to testing objectives.

Electron Microscopy

Negative-contrast EM and immuno-EM have provided rapid and valuable information on virus structure and antigenic reactivity during primary virus isolation.³⁶

Polymerase Chain Reaction and Sequencing

Diagnostic PCR assays for HeV and NiV are in routine use at the Australian Animal Health Laboratory (Geelong) and the U.S. Centers for Disease Control and Prevention (Atlanta). The ability to select primer sets for particular genes allows test sensitivity and specificity to be tailored to testing objectives. The technique may be used as a primary diagnostic tool to

detect viral sequences in fresh or formalin-fixed tissue and as an adjunct to virus isolation to characterize virus isolates rapidly.²²

Serum Neutralization Tests

The SN test is regarded as the “reference standard” serologic test for Hendra and Nipah viruses. Sera are incubated with live virus in microtiter plates to which Vero cells are added, and cultures are read at 3 days.¹⁹ The use of live virus means that SN tests should only be performed in a PC4 facility.

Enzyme-Linked Immunosorbent Assay

The ELISA tests provide a rapid, inexpensive, and safe means of conducting serologic investigations. Indirect ELISAs have been developed for the detection of anti-Nipah and anti-Hendra immunoglobulin G (IgG), and a capture ELISA has been developed for detection of anti-Nipah IgM.¹⁹ Currently available ELISA tests still need to combine excellent sensitivity and specificity with respect to SN results. Further improvement of ELISAs or other serologic tests is required for future epidemiologic studies of HeV and NiV in bats. Recent advances in the development of multiplexed microsphere assays show particular promise in this area.

DISEASE ECOLOGY AND SPILLOVER MECHANISMS

The reasons for the emergence of these zoonotic bat-borne viruses in recent years are yet to be resolved. Although not yet established, it has been hypothesized that changes in bat ecology are driving disease emergence in these species.²⁵ Flying foxes are particularly vulnerable to habitat loss or modification resulting from the ephemeral nature of their food resources.²¹ Land use change has resulted in population decline, population concentration during resource scarcity, distributional changes, and urbanization of flying fox populations throughout the Old World Tropics.^{21,28,47} These processes could lead to disease emergence either by changes in viral dynamics or by increased contact with domestic animals and humans.

Hendra and Nipah viruses appear to be ancient viruses that co-evolved with and are well adapted to their natural flying fox hosts.^{27,48} The emergence of these viruses in humans has required a bridge from the natural host to a susceptible “spillover” host. Such



Fig 28-6 Gray flying fox (*Pteropus griseus*), East Timor. (See Color Plate 28-6.) (Courtesy Andrew C. Breed.)

bridges typically result from changes to the agent, the host, or the environment. The close RNA sequence match among flying fox, livestock, and human isolates of Hendra and Nipah viruses suggests that emergence is more likely associated with ecologic changes that have promoted contact between bats and livestock, rather than with genetic change leading to increased virulence.⁴²

Available data on many flying fox species suggest that populations in Australia and Southeast Asia are declining, with disruption occurring throughout their range (Figures 28-6 and 28-7). In Southeast Asia, anthropogenic activities (primarily habitat destruction and hunting) constitute the major threats. Deforestation, whether for agricultural land, commercial logging, or urban development, is widespread and results in loss or abandonment of roosting sites and loss of feeding habitats. This habitat loss caused by clearing is often exacerbated by tropical storms because the remnant forest may be particularly prone to high-wind damage. Hunting, whether for consumption, sport, or crop protection, at both a local and a commercial level, results in the abandonment of roost and feeding sites.⁴⁷ A scenario thus emerges of flying fox populations under stress with altered foraging and behavioral patterns, of niche expansion, and of closer proximity to humans. In Australia the geographic redistribution of roosting sites has been increasingly into urban areas in recent decades.²⁸

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Fig 28-7 Global distribution of flying foxes (genus *Pteropus*). The sites of disease outbreaks caused by henipaviruses are indicated by asterisks. (See Color Plate 28-7.) (Eaton BT, Broder CC, Middleton D, Wang L: *Hendra and Nipah viruses: different and dangerous*, Nat Rev Microbiol 4:23-35, 2006, Macmillan Magazines.)

RESERVOIR HOST MANAGEMENT STRATEGIES

The sporadic and apparently rare nature of HeV spillover events from flying foxes to horses, the low infectivity for horses (and thus limited economic impact), and the apparent absence of direct transmission from flying foxes to people have resulted in more emphasis on management strategies for horses than flying foxes. Quarantine of infected premises, movement controls on stock, and disinfection have so far proved effective.⁷ Veterinarians involved in these disease investigations are advised to wear appropriate protective equipment and to use a limited necropsy approach, because horses have been the source of infection for all four human cases. Putative risk factors for infection in horses appear to be age (>8 years old), breed (Thoroughbred), housing (paddocked), season (late gestation or birthing season of local flying fox populations), and the presence of food trees favored by flying foxes in the index-case paddock.²³ A considerable research focus on the ecology of HeV has yet to define the route of virus excretion or any temporal pattern of infection in flying foxes. This information and knowledge of the actual mode of flying fox-to-horse transmission would facilitate a risk management approach to spillover infection in horses.

In marked contrast to HeV, the NiV outbreak in peninsular Malaysia in 1999 had an enormous economic and social impact.⁵⁰ Nipah virus was highly infectious for pigs, with all age and sex classes susceptible. The pattern of on-farm infection was consistent with respiratory transmission; between-farm spread was generally associated with the movement of pigs. Human

infections were predominantly attributed to contact with live pigs; none was attributed to contact with bats.¹⁵ Horizontal transmission was not a feature of infection in humans. Recommended host management strategies primarily targeted pig-to-pig transmission.²⁴

Although strategies directed at the flying fox-pig interface are limited by the incomplete knowledge of the ecology of NiV, several simple on-farm measures may be taken to reduce the likelihood of spillover events. The removal of fruit orchards and other food trees favored by flying foxes from the immediate vicinity of pig farms greatly reduces the probability of flying fox-pig contact. Similarly, the wire screening of open-sided pig sheds is a simple and inexpensive strategy to prevent direct contact between flying foxes and pigs. Indirect contact (with flying fox urine or feces or partially eaten fruit) may be avoided by ensuring roof runoff does not enter pig pens.¹⁴

Henipavirus spillover to domestic animals may be effectively controlled by the methods previously mentioned, but events in Bangladesh warn against complacency in elimination of the zoonotic risk of henipaviruses using these methods alone.

A study has shown that an oral vaccine was capable of inducing a protective immune response to rabies in vampire bats after oral vaccine delivery, and therefore an oral vaccination approach may be plausible for other bat species.⁵⁹

Other authors discuss the possibility of using an oral vaccine for henipaviruses in flying foxes in the future.⁴⁵ They observe that the presence of antibodies to Hendra and Nipah viruses in healthy flying foxes could warrant the inclusion of a biomarker in a vaccine to distinguish between vaccinated individuals

and naturally infected individuals. However, they also caution that various aspects of flying fox behavior require further study before development of an oral vaccine strategy.

Development of a vaccine for HeV or NiV to be used in wild flying fox populations is not likely to occur in the near future. However, a better understanding of flying fox behavior and ecology, henipavirus dynamics in flying foxes, and anthropogenic factors that facilitate spillover events will offer cost-effective and practical solutions for preventing future outbreaks.

CONCLUSION

The evident horizontal human transmission and the apparent absence of an intermediate domestic animal reservoir in the Bangladesh outbreaks of Nipah virus are disturbing epidemiologic features that highlight the potential for change in viral transmission dynamics and the urgent need for detailed study of bat paramyxoviral ecology and increased understanding of spillover mechanisms.²⁴ Also, given that four of the six paramyxoviruses known to naturally infect bats have been identified within the last 15 years, and that the vast majority of bat species have never been surveyed for evidence of paramyxoviral infection, there may well be other, currently unidentified paramyxoviruses in wild bat populations.

To understand fully the factors that drive disease emergence, we must attempt to understand these viruses and their hosts at a range of spatial scales. We currently know a considerable amount about the molecular biology of the viruses discussed,⁶⁵ little about the interaction between the viruses and their hosts,^{68,69} and even less about the biology of the viruses at the level of the host population.^{8,25}

Bats play vital roles in pollination, seed dispersal, and insect predation in the ecosystems where they occur⁵²; they must be conserved to maintain ecologic health and biodiversity. The increasing anthropogenic encroachment on and change in these ecosystems will test our ability to assess and manage effectively the risk posed by the pathogens harbored by bats and other wildlife species.

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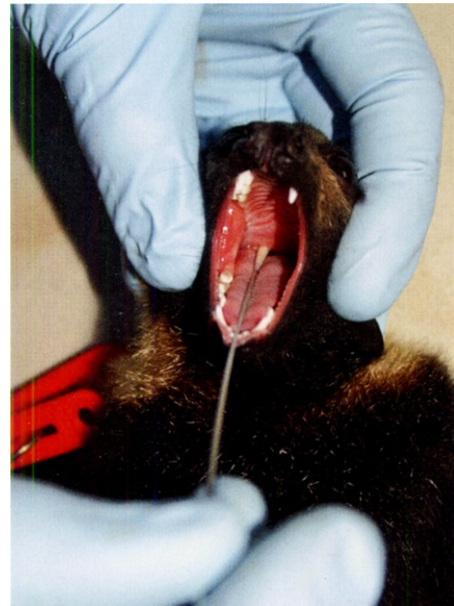
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Color Plate 28-1 Administration of oral rehydration solution to greater flying fox (*Pteropus neohibernicus*) after general anesthesia for application of satellite collar in Papua New Guinea. (For text mention, see Chapter 28, p. 226.) (Courtesy Andrew Breed.)



Color Plate 28-3 Anesthesia of wild-caught, spectacled flying fox (*Pteropus conspicillatus*), using isoflurane and oxygen, for Hendra virus surveillance in North Queensland, Australia. (For text mention, see Chapter 28, p. 228.) (Courtesy Dr. Jack Shield.)



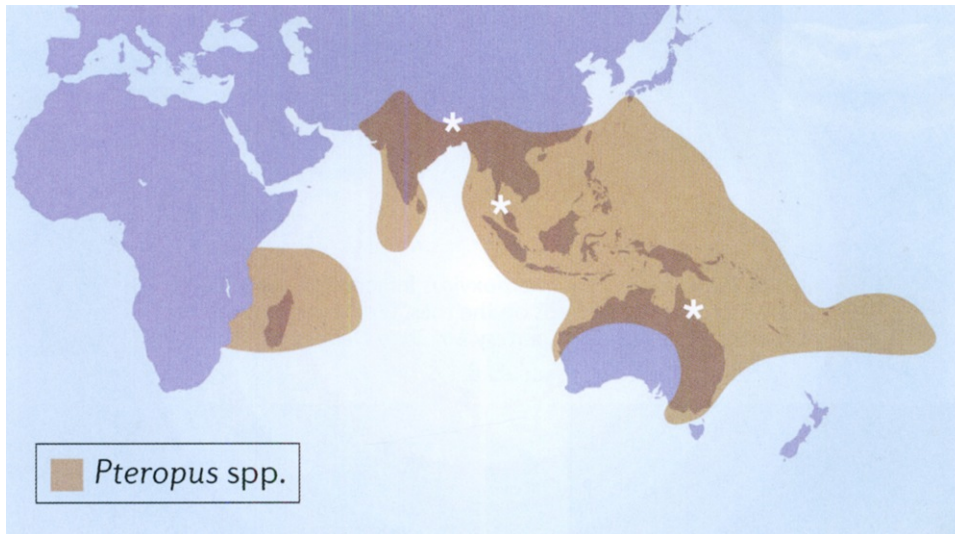
Color Plate 28-4 Collection of oral swab from anesthetized spectacled flying fox (*Pteropus conspicillatus*) for Hendra virus antigen detection. (For text mention, see Chapter 28, p. 228.) (Courtesy Dr. Jack Shield.)



Color Plate 28-5 Collection of piece of wing membrane from anesthetized spectacled flying fox (*Pteropus conspicillatus*) for molecular genetic studies. This technique is used to elucidate population structure of flying fox species for henipavirus epidemiologic studies. (For text mention, see Chapter 28, p. 229.) (Courtesy Dr. Jack Shield.)



Color Plate 28-6 Gray flying fox (*Pteropus griseus*), East Timor. (For text mention, see Chapter 28, p. 231.) (Courtesy Andrew Breed.)



Color Plate 28-7 Global distribution of flying foxes (genus *Pteropus*). The sites of disease outbreaks caused by henipaviruses are indicated by asterisks. (For text mention, see Chapter 28, p. 232.)

CHAPTER 29

Medical Aspects of Red Squirrel Translocation

ANTHONY W. SAINSBURY

This chapter is based on work in the United Kingdom (U.K.) on the translocation of red squirrels (*Sciurus vulgaris*) for conservation purposes. The other species of squirrel present in the U.K. is the grey (gray) squirrel (*Sciurus carolinensis*), an alien species introduced from the United States in the nineteenth century. Both these species are diurnal tree squirrels (order Rodentia; subfamily Sciurinae). There is no attempt to cover translocation for rehabilitation purposes, already described in Sainsbury,²⁴ although many of the principles are the same.

BIOLOGY

Both the red and the gray squirrels inhabit conifer and broadleaf forests, as well as urban parks and gardens with mature trees. They are solitary for much of the time, but communal nesting may occur during winter and spring. Dominance hierarchies are not dependent on gender; larger and older animals are more dominant.¹⁴ Red squirrel densities are lower (0.3-1.0 squirrel per hectare [ha]) than gray squirrels in broadleaf woods (2-8 squirrels/ha) but tend to be similar in conifer woods (0.03-1.3 squirrels/ha) (1 ha = 2.47 acres). Aggressive encounters within species are rare but may result in bites to the ears, dorsum, rump, or tail. Encounters between red and gray squirrels are usually amicable.³⁴ Scent marking occurs on specific branches or tree trunks using urine and possibly secretions from mouth glands by face-wiping behavior.

Dispersal of juveniles and some adults principally occurs during the autumn and occasionally at other times of the year. These squirrel species do not hibernate and are active all year, although they may remain in their nest (drey) for 2 or more days during severe winter weather.¹⁴ There is an annual cycle of numbers, with a peak after breeding in the autumn, overwinter losses, and a low point in spring before recruitment.

The diet of free-living red or gray squirrels consists principally of tree seeds, such as hazelnuts, beech mast, acorns, and conifer seed, as well as fruits, berries, and fungi. Other foods include buds, shoots, flowers, bark, invertebrates, and lichen.¹⁴ There are reports of squirrels eating bones found in their environment^{2,4,7} and in captivity.⁹ Feeding signs for squirrels include hazelnuts split open, leaving two pieces of shell with clean edges; characteristic "cores" of conifer cones, with associated piles of stripped scales with clean-cut edges (rather than the ragged edges made by birds); and bark stripping.¹⁴

UNIQUE ANATOMY

Red squirrels weigh between 270 and 320 g when adult; gray squirrels are heavier and weigh between 500 and 600 g. The body weight may increase in the autumn by as much as 10% in red squirrels and 17% to 13% in gray squirrels.¹⁴ Females have four pairs of nipples. The scent glands are present at the commissure of the mouth and in the upper and lower lips.

As with many other rodents, the genders may be differentiated by the distance between the genital opening and the anus, which is short in females and about 10 mm in adult males. The reproductive tract regresses in the autumn and perhaps in the winter if food supplies and weather are poor.¹⁴ The feces are cylindrical or round, slightly smaller than those of rabbit (8 mm in diameter), and dark gray to black but vary according to the diet.¹⁴

Dentition

Incisors in squirrels, as in other rodents, grow continuously, and the lower incisors in particular occupy long sockets. The incisors have an enamel coating on

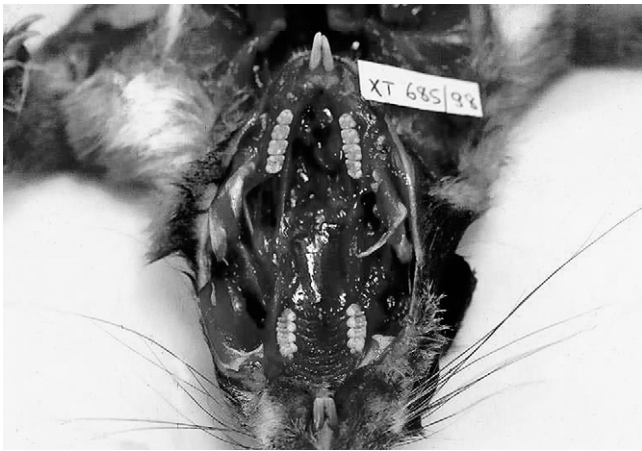


Fig 29-1 Oral cavity of young adult red squirrel. Note the cusps and ridges of the cheek teeth and the chisel-shaped incisors. (See Color Plate 29-1.) (Courtesy Mr. Terry Dennett.)

the full length of their labial surfaces, whereas at the buccal aspect only the softer dentin is present, so the incisors are worn to a chisel-shaped cutting edge (Figure 29-1). The dental formula in both red and gray species follows; the first upper premolar is rudimentary and vestigial.¹⁴

1	0	2	3	
Incisors	+ Canines	+ Premolars	+ Molars	= 11
1	0	1	3	

Red squirrels' lower incisors erupt at 19 to 21 days of age and the upper incisors at 31 to 42 days.¹⁴ The cheek teeth (molars and premolars) erupt from 7 weeks of age onward, and by 10 weeks all the cheek teeth are present. Primary first lower and only the second upper premolars are shed at 16 weeks and are replaced by permanent teeth. There are no canine teeth, and a diastema exists between the incisors and the cheek teeth.²⁴ The cheek teeth are quadrate with rounded, blunt, cone-shaped, bunodont marginal cusps and a concave central area (fossa) on their occlusal surfaces (see Figure 29-1). The occlusal surfaces of the upper cheek teeth are traversed by weak, transverse ridges.¹⁴ A young squirrel has a layer of enamel covering the surface of each cheek tooth, including the cusps and ridges. This layer becomes worn with age, exposing the underlying dentin.²⁹

RESTRAINT AND HANDLING

Physical Restraint

Red squirrels are prone to breath holding when handled and must be restrained gently, quietly, with speed, and only for short periods. When breath



Fig 29-2 Baited trap suitable for capturing red squirrels, under license. (See Color Plate 29-2.)

holding, the squirrel becomes immobile and has a fixed stare. Breath holding may in turn cause the squirrel to develop hypoxia, hypercapnia, and bradycardia, which appears to be fatal in some cases. On encountering this response, the squirrel should be placed immediately in a dark box or bag and allowed to recover unaided.

Both red and gray squirrels may inflict deep bites with their incisor teeth and possibly transmit zoonotic agents, so it is advisable to wear gloves when handling them. Although the bite of a squirrel may penetrate leather gauntlets, the use of greater protection may make handling difficult. Aids for restraint include the use of a net, a "squeeze" cage, a sack, or a wire handling cone.

A variety of live traps are available to capture squirrels. Red squirrels are best captured in a single-capture trap with a removable nest box attached¹⁶ (Figure 29-2). Gray squirrels, on the other hand, may be trapped in multicapture cage traps.¹⁵ A license may be required to trap squirrels. Red squirrel traps are prebaited with apple, carrot, corn, peanuts, sunflower seeds, and hazelnuts for up to a week before setting. Gray squirrel traps are usually baited with whole corn.

One method to remove the trapped squirrel is to encourage it to leave the nest box or trap and enter a burlap (hessian) sack. For example, the mouth of the sack may be closed firmly around the nest box, with the lid of the box removed at the same time. Most squirrels will voluntarily enter the sack, then may be confined to a corner. The physical form of the squirrel may be detected through the sack, and the squirrel may be safely restrained by placing downward pressure on the dorsum, using the thumb and forefinger to control the head and neck and the remainder of the hand to control the body. The sack's mouth may then be reflected to examine the squirrel and, if necessary, apply a malleable rubber face mask to induce anesthesia.



Fig 29-3 Handling cone used for restraint of squirrels. (See Color Plate 29-3.) (Courtesy Dr. Peter Lurz.)

Alternatively, squirrels may be transferred from a nest box to a suitably sized handling cone constructed of wire mesh (230 mm long and 20 mm in diameter at mouth of cone), in which they may be examined or anesthetized using a face mask (Figure 29-3). The squirrel will voluntarily run into the cone and may then be prevented from reversing by placing a finger behind it.

Chemical Immobilization

Ketamine at approximately 40 mg/kg body weight by intramuscular (IM) injection will provide sedation that wanes over approximately 1 hour. Flecknell¹² found that a combination of medetomidine (0.5 mg/kg) and ketamine (75 mg/kg) administered in the same syringe by intraperitoneal injection provided effective anesthesia (although not necessarily for major surgery) in rats. Routh²² used this combination of agents by IM injection in gray squirrels. Partial reversal of anesthesia with atipamezole (1 mg/kg subcutaneously) is possible. (In rats, this should not be attempted until 20 minutes or more after induction because of the undesirable effects of ketamine.)

ANESTHESIA

As with other rodents, squirrels do not vomit and so it is not necessary to starve them prior to anesthesia. It may be valuable to administer fluids to squirrels under anesthesia, by the subcutaneous route in well hydrated animals, to compensate for losses through respiration and urination.

As in all small mammals with a high surface area/body weight ratio, additional heat may be needed for red squirrels during anesthesia to maintain body temperature. Anesthesia may be safely achieved using

isoflurane at 1% to 4% in oxygen administered by a malleable face mask. Intubation is difficult, but Flecknell's method¹² for rats could be used as follows: the squirrel is positioned in dorsal recumbency and the tongue pulled gently forward and to one side. The larynx is visualized with a purpose-made laryngoscope.⁵ The larynx may then be intubated with an intravenous cannula (12-16 gauge) using a suitable speculum. A small piece of rubber tubing or some Micropore tape (3M, Loughborough), positioned around the catheter about 5 to 10 mm from its tip, will prevent a bronchus being entered or leakage of gas around the tube, making ventilation more effective.

Inhalation anesthesia may be used effectively in the field to anesthetize squirrels, which is necessary for examination during a translocation. A portable vaporizer, small cylinder of oxygen, and a malleable face mask constitute the necessary kit for field anesthesia.

On recovery, squirrels are best returned to a solid-sided wooden box with bedding material and an access port to monitor the animal. The squirrel may be wrapped in kitchen foil or bubble wrap to reduce loss of body heat.

DISEASE RISK ANALYSIS

Given the disease risks associated with translocations,¹⁸ a risk analysis must be undertaken before planning a squirrel translocation, as for any species of living organism. Davidson and Nettles⁸ and Leighton¹⁹ set out the broad principles of undertaking such an analysis, which include the following:

1. Gathering data on the infectious agents possessed by the animals to be translocated, conspecifics at the translocation site, and other species at the translocation location, through literature review and diagnostic testing.
2. Evaluating the risk that novel host-parasite encounters caused by the translocation might result in disease in any of these species.
3. Assessing the risk that the translocation might result in artificial intensification of any existing infectious agents and therefore give rise to disease.

The most serious disease risk from a translocation is from the transfer of an alien pathogen to a naive population, which has the potential to cause an epidemic of disease. Several catastrophic epidemics have arisen in this manner.¹⁸

Infectious agents that should be considered when translocating squirrels include squirrel poxvirus (SQPV),

adenovirus,²³ *Salmonella* spp., *Campylobacter* spp.,¹¹ *Yersinia* spp., *Brucella* spp. (*Francisella tularensis* in some parts of Europe and North America), and *Leptospira* spp.²³ Red squirrel translocation to an area where gray squirrels are present is not recommended given current knowledge of the epidemiology of SQPV. Red squirrels might contract SQPV from gray squirrels and develop epidemic disease.³⁰ The SQPV status of free-living gray squirrels at the translocation site could influence a decision on the release of red squirrels into an area inhabited by gray squirrels. If gray squirrels at the translocation site are found to be seronegative and apparently have not been exposed to the virus, the risk of red squirrels contracting SQPV infection is reduced.

Squirrel Poxvirus

Squirrel poxvirus is the etiologic agent of a disease known to cause high mortality in red squirrels.³¹ There is good evidence that the gray squirrel is a reservoir host of SQPV,²⁷ and only a single case of disease associated with a parapox-like virus has been recorded in a gray squirrel.¹⁰ It is unclear how the SQPV is transmitted between squirrels, but direct or indirect skin-to-skin or skin-to-body fluid contact may be involved. The SQPV produces characteristic skin lesions in red squirrels: erythematous exudative dermatitis and ulceration, with some lesions covered by hemorrhagic crusts,²⁵ especially on the face, ventral skin surfaces of the body, medial skin of the legs, and the genital region (Figures 29-4 and 29-5).

The disease may be diagnosed by electron microscopy (EM) of skin lesions. The clinical signs may be less severe in some cases, and evidence indicates that red squirrels show a variable immune response to the virus, and that some may survive the disease, despite showing the clinical signs for up to 4 weeks.³¹ These cases may benefit from supportive therapy, such as antibiotics, antifungal agents, analgesics, and fluids. Hand feeding may be required if infections of the conjunctiva prevent vision. No vaccine is available to prevent SQPV disease.

Adenovirus-Associated Disease

Diarrhea, splenic necrosis, and mortality have been associated with adenovirus infection of the intestine in captive and free-living red squirrels found dead.^{6,23} The large intestinal contents had a characteristic gray, pasty appearance in most cases described, and adenovirus was detected by EM. The pathogenicity of ade-



Fig 29-4 Squirrel poxvirus infection in red squirrel, showing lesions in the facial area. (See Color Plate 29-4.) (Courtesy Mr. Terry Dennett.)



Fig 29-5 Squirrel poxvirus infection in red squirrel, showing ulcerative lesions on the toes. (See Color Plate 29-5.) (Courtesy Mr. Terry Dennett.)

novirus in red squirrels has not been confirmed, and the geographic distribution, source of the virus, and prevalence of infection are unknown.

Bacterial Infections

Several bacterial agents have been reported to cause disease in squirrels, as previously noted, but no specific studies have investigated the epidemiology of these infections. Therefore, for the purposes of undertaking a disease risk analysis, the epidemiology should be assumed to be similar to the situation in other mammals.

QUARANTINE

Assuming a disease risk analysis indicates that the benefits of translocation outweigh the disease and

other risks, the animals to be translocated (if captive bred) should be placed into quarantine to prevent the acquisition of new infectious agents during the period before translocation. Screening for infectious agents is advisable at this stage.

Red squirrels may be tested for antibodies to SQPV using an ELISA,²⁷ and seropositive red squirrels might be considered for release into an area where seropositive gray squirrels are present. Fecal examination for endoparasites, fecal bacteriology, upper respiratory tract bacteriology, blood smear examination for hemoparasites, and an examination for ectoparasites should also be performed.

If detected, parasites must be identified, wherever possible, to determine whether or not they are alien to the release site.

HEALTH EXAMINATION BEFORE RELEASE

Many authors advocate a detailed health examination before translocation.³⁶ Red and gray squirrels may only be examined effectively if under anesthesia. Examination of the oral cavity is particularly important to ensure that the teeth are not overgrown and that they occlude satisfactorily. Three of 91 free-living red squirrels examined in one study showed oral disease.²⁶ The most common oral lesions were malocclusion of the incisor teeth (4 of 364 red squirrels) and attrition of the cheek teeth.

The health examination should ideally include hematologic analysis, serum/plasma chemistry profile, urinalysis, and abdominal palpation to assess for impaction and lesions of a chronic infectious origin such as amyloidosis. Body condition may be assessed as thin, good, or fat by palpation of the soft tissues surrounding the femur.

Findings that probably prevent successful translocation include incisor or molar overgrowth, malocclusion, insufficient function of the organs of sight or hearing, and any disability that might permanently affect the squirrel's ability to climb or balance. Pregnant or lactating squirrels should be returned to source as soon as possible. Released red squirrels should be marked. Subcutaneously implanted microchips or Dalton Mini Rototags applied to both ears may be used. Radio-tracking released squirrels improves the ability to monitor their well-being; radio collars may be fitted while the squirrel is under anesthesia. A subcutaneous injection of 2% to 4% of body weight with lactated Ringer's or Hartmann's solution is recom-

mended to compensate for body fluid loss during transit and anesthesia.

DISEASE CONTROL

If alien parasites are unidentified, a decision must be made whether to proceed with the translocation. Options include canceling the movement if the risks appear too high or attempting to eliminate the infection through treatment and then retesting. Pre-release immunization with a killed rabies vaccine is recommended if squirrels are to be released into an area where rabies might occur.

Anthelmintics or other parasiticides should be administered to eliminate alien parasites and may be given to control other infestations. Appropriate pyrethrin-type or pyrethroid acaricides and an avermectin should be applied for the elimination of ectoparasites to reduce the possibility of tularemia (*F. tularensis*) infection. Consideration should be given to the administration of antibiotics to reduce the severity of upper respiratory tract infections based on nasal culture and sensitivity testing.

Although *Leptospira* spp. have rarely been isolated from squirrels,²¹ all rodent species should be considered as carriers of *Leptospira* and may serve as sources of infection for other animals and humans. The risk of transmitting leptospirosis to other animals at the release site should be considered.

RELEASE TECHNIQUES

The favored time of year for translocation is August to November, when squirrel populations have finished breeding, and this is a usual time for dispersal and social reorganization. Furthermore, the weather is not cold or unduly wet in the U.K. and tree seed availability tends to be good at this time of year.

Several studies have reported on the release of captive-bred or translocated wild squirrels.^{3,13,17,20,35} Venning et al.³³ suggested that proximity to roads should be avoided when releasing squirrels because of the high probability of road traffic killing dispersing squirrels. Kenward and Hodder¹⁷ carried out a soft release of 14 red squirrels in Dorset from 3.4-m³ cages, in which they were held for 3 to 6 days.¹⁷ Ten squirrels died within 45 days, and all were dead by 126 days after release. In southeastern Scotland, Pritchard and Bruemmer²⁰ reported that 7 of 44 red squirrels in a release program died before release, 9 died after

release from cages measuring $4 \times 4 \times 2$ m, in which the squirrels were housed for 10 to 20 days, and "two or three" had emigrated from the 280 ha of release-site woodland. The fate of the others was not known.

Venning et al.³³ reported the successful translocation of free-living red squirrels for conservation purposes, with greater than 75% survival 2 weeks after release and greater than 50% survival up to the following breeding season 6 months later.³³ A soft-release method was adopted by Venning et al.³³ using a 1-ha pre-release pen in Thetford Chase, East Anglia. Each squirrel that had been translocated from elsewhere was placed on its own in a nest box, attached to a tree 3 to 4 m (10-13 ft) above ground level in the pre-release pen. Each box contained wood-shavings bedding and some food (apples, carrots, corn, peanuts, sunflower seeds, wheat, hazelnuts), and the entrance to each box was loosely plugged with shavings to prevent the squirrel from immediately bolting. Food and water, containing a calcium supplement, were placed on tables within the pen. The boxes were checked 6 to 9 hours later, and if still in place, the shavings plugs were removed. Four hours later, any squirrels remaining in their box were flushed out. The squirrels were housed in the pre-release pen for 4 to 6 weeks before release. Twenty food hoppers were available in the forest for squirrels to use within 400 m (440 yd) of the pen. After leaving the pen, the squirrels were monitored by radio tracking and live trapping.

Usher-Smith³² found that 6 of 10 rehabilitated orphan red squirrels survived for a year after a soft release using portable release cages measuring $720 \times 580 \times 1350$ mm high, wired to trees approximately 0.7 m ($2\frac{1}{3}$ ft) off the ground, and for $3\frac{1}{2}$ months the squirrels could return to these release cages.

Two hard-release translocation studies have been carried out in continental Europe, one in an urban park in Antwerp, Belgium,³⁵ and one in woodland in Italy.¹³ In neither target area were red or gray squirrels present. Fornasari et al.¹³ released eight red squirrels, of which four remained alive after 2 months, and a population of red squirrels was present 8 years later.¹³ In the Belgium study,³⁵ nest boxes were provided in the park and 19 red squirrels were released on the same day of capture from three different source areas. Eight of the 19 squirrels (three males and five females) survived to breed. In contrast, Adams et al.¹ found that of 38 gray squirrels (*Sciurus carolinensis*) hard-released in Maryland (U.S.), 37 died or disappeared from the release area within 88 days.

These examples illustrate how difficult the release of squirrels may be, but that it is more successful when

they are given several weeks to acclimatize in soft-release pens. As a result of the work involved in a red squirrel release project, it may be better to release more than one animal at one time.

The presence of domestic dogs and cats in the release area may reduce the chances of successful releases. Red squirrels may be released successfully into both conifer and deciduous forests.

POSTRELEASE SURVEILLANCE

Telemetry is the preferred method to monitor squirrels because both sick and dead animals may be located. Radio-tracking devices have a limited life span, so this type of monitoring is likely only possible for a few weeks after release. Traps should be set in the vicinity of the release site to allow direct examination of the released squirrels and assessment of their health status. The ease with which they may be trapped will depend on natural food supplies and the degree to which the squirrels disperse after release.

Detailed health examinations should be performed on trapped animals. Arrangements will need to be made to hospitalize any sick squirrels. Any dead animals will require detailed postmortem examination to determine cause of death so that changes can be made in the management of the squirrels, as necessary, to improve their welfare and survival.

CONCLUSION

Translocation of red squirrels in the U.K. for conservation purposes is not a viable exercise at this time because of the presence of gray squirrels, presumed to be carriers of SQPV in almost all areas where red squirrels have declined or now are extinct. Gray squirrels are extending their geographic range, and no effective methods currently exist for protecting red squirrels. However, effective methods have been developed to undertake translocation and may be valuable in the future and to conservationists in other parts of the world.

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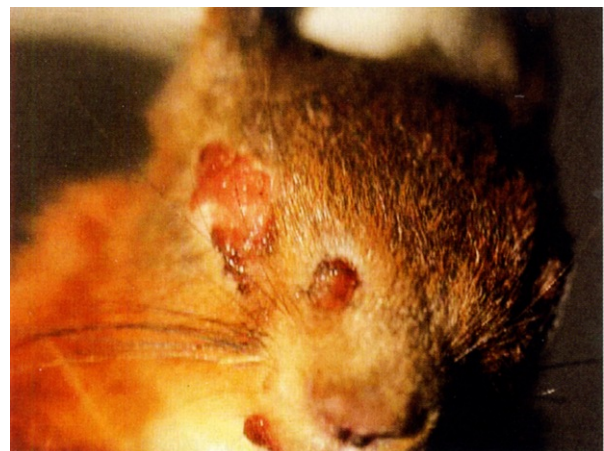
Color Plate 29-1 Oral cavity of young adult red squirrel. Note the cusps and ridges of the cheek teeth and the chisel-shaped incisors. (For text mention, see Chapter 29, p. 237.) (Courtesy Mr. Terry Dennett).



Color Plate 29-2 Baited trap suitable for capturing red squirrels, under license. (For text mention, see Chapter 29, p. 237.)



Color Plate 29-3 Handling cone used for restraint of squirrels. (For text mention, see Chapter 29, p. 238.)



Color Plate 29-4 Squirrel poxvirus infection in red squirrel, showing lesions in the facial area. (For text mention, see Chapter 29, p. 239.) (Courtesy Mr. Terry Dennett).



Color Plate 29-5 Squirrel poxvirus infection in red squirrel, showing ulcerative lesions on the toes. (For text mention, see Chapter 29, p. 239.) (*Courtesy Mr. Terry Dennett*).

CHAPTER 30

Neuroleptics in Great Apes, with Specific Reference to Modification of Aggressive Behavior in a Male Gorilla

SHARON P. REDROBE

The use of drugs to treat animal behavioral problems is a relatively new field of veterinary medicine. Most reports of using these drugs in zoo animals are limited to ungulates,² with few describing their use in great apes.^{3,5,6,9} One report concludes that psychoactive drugs have not been successful in great apes when used to curb aggression, although this outcome may have been the result of misdiagnosis, inappropriate dose rates, or insufficient treatment duration.⁶

When using drugs to moderate or change behavior, it is important to realize the limitations of medical therapy. Drug selection should be based on a careful behavioral assessment, and the animal should be monitored for side effects of the drugs. Also, many of the drugs that may be used in this area have the potential for human abuse, so their prescription and use should be carefully controlled.

Drugs alone are unlikely to be successful in producing long-lasting behavioral changes unless they are used in conjunction with a behavioral modification program. Therefore, teamwork among the veterinarian, the animal keepers and animal behaviorists, trainers, and human medical professionals is essential to ensure a successful outcome.

CATEGORIES OF NEUROLEPTICS AND ANTIDEPRESSANTS USED FOR BEHAVIORAL MODIFICATION

Neuroleptics, also referred to as *antipsychotics* in human medicine, include butyrophenones (haloperidol, azap-

erone), phenothiazines (perphenazine, fluphenazine), thioxanthenes (flupenthixol, zuclopenthixol), and substituted benzamides (sulpiride). These drugs cause a range of degrees of sedation, alpha-adrenoceptor blocking activity, extrapyramidal symptoms, and antimuscarinic effects.¹ These drugs generally tranquilize without affecting consciousness or excitement, but should not be regarded merely as "tranquilizers." In humans, for the short term, they are used to calm disturbed patients, whatever the underlying psychopathology. Newer neuroleptics, such as risperidone, also called *atypical* antipsychotics, may be better tolerated because extrapyramidal symptoms occur less frequently (in humans).

Antidepressants may also be used to moderate abnormal animal behaviors, particularly the selective serotonin reuptake inhibitors (SSRIs), such as citalopram and fluoxetine (Prozac; Elly Lilly, U.S.A.), and the monoamine oxidase inhibitors (MAOIs), such as clomipramine.⁸ Interaction between these two groups may complicate switching from one drug to another; MAOIs are rarely used in human medicine because of the dangers of dietary and drug interactions. Other antidepressants should not be started for 2 weeks after treatment with MAOIs has stopped (3 weeks with clomipramine). Conversely, an MAOI should not be started until at least 2 weeks after antidepressant (3 weeks with clomipramine) has stopped. For this reason, the selection of SSRIs or MAOIs for the treatment of zoo animals should be undertaken with great care because if one is not working, the time required to change drugs is prolonged, which may lead to an exacerbation of the welfare issue.

CAUTIONS, CONTRAINDICATIONS, AND SIDE EFFECTS OF NEUROLEPTICS

Neuroleptics should be avoided in patients with renal or hepatic impairment or cardiovascular disease. They are best avoided during pregnancy. Withdrawal after long-term therapy should always be gradual and carefully monitored to prevent acute withdrawal syndromes or rapid relapse. Antimuscarinic effects are a side effect of most neuroleptics and include dry mouth and constipation.

The most significant side effects are the extrapyramidal signs. These effects occur most often with the piperazine phenothiazines (perphenazine, fluphenazine), but also with the butyrophenones (haloperidol, azaperone). The phenothiazine group may be further divided in groups 1, 2, and 3. Group 3 phenothiazines include perphenazine, which is widely used in zoo animals, particularly ungulates,² because it is associated with fewer sedative effects than the other groups. However, perphenazine may produce more pronounced extrapyramidal effects. Extrapyramidal signs are easy to recognize but cannot be predicted because they depend on dose, type of drug, and individual susceptibility. Extrapyramidal signs include parkinsonian-like symptoms (including tremor), dystonia (abnormal face and body movements), akathisia (restlessness), and tardive dyskinesia (involuntary rhythmic movement of tongue, face, and jaw). The latter usually develops in humans who receive long-term therapy but may occur on short-term treatment and low doses or after withdrawal of the drug.

Neuroleptic malignant syndrome (hyperthermia, fluctuating level of consciousness, muscular rigidity, and autonomic dysfunction with pallor, tachycardia, labile blood pressure, sweating, and urinary incontinence) is a rare but potentially fatal side effect. Discontinuation of the drug is essential because no specific treatment exists for this syndrome, which usually lasts 5 to 7 days after cessation of therapy in humans.

Other side effects include drowsiness, insomnia, convulsions, dizziness, gastrointestinal disturbances, cardiovascular disturbances including sudden death, photosensitization, and corneal and lens opacities. Therefore, patients receiving any of these drugs should be carefully monitored by staff who are aware of the side effects of these drugs and who will ensure that any such signs are reported to the veterinarian as a matter of urgency.

Drug selection in human medicine is based on the degree of sedation required and the patient's suscepti-

bility to extrapyramidal effects. This susceptibility is generally unknown when dealing with great apes. Prescribing more than one antipsychotic at a time is not recommended unless under close medical supervision; this may increase the risks, and there is no evidence that side effects are minimized. Given the lack of data in great apes, a number of regimens will likely be tried before one suitable for the particular patient and condition is found. In particular, care should be taken to select the drug regimens in a certain order to avoid potentially dangerous drug interactions.

Therefore these drugs should be carefully selected for use in great apes because they do not pose a simple and safe solution to the behavioral management of zoo animals. When used carefully, however, neuroleptics may provide an extra tool for managing difficult patients who are unresponsive to behavioral therapy alone.

USE OF NEUROLEPTICS IN GORILLAS

Few published reports on the use of neuroleptic or behavior-modifying drugs in great apes exist. A survey on the use of psychoactive drugs in great apes included the use of haloperidol, with and without fluoxetine, or risperidone to control aggression in male gorillas.⁶

A case study on the control of aggression and abnormal behaviors in a group of two female gorillas and one male gorilla described the use of haloperidol and thioridazine in all three animals.⁵ Another paper has described the use of haloperidol in a female gorilla to treat self-mutilation.³ Zuclopenthixol has been used to reduce anxiety without sedation in a group of 10 gorillas transported by air from Europe to Australia.¹²

Perphenazine enanthate as a long-acting injectable product has been used to moderate aggression in an adult male gorilla intermittently over several months. On one occasion an extrapyramidal side effect similar to neuroleptic malignant syndrome was noted 3 days after injection, characterized by a hypertonic crisis five times in 1 hour.⁷ Oral zuclopenthixol has been used in a gorilla reacting aggressively to visiting public, using doses of 10 to 25 mg three times a day. The dose was gradually tapered to zero, with a decrease of 5 mg every week.⁷ Transportation of an adult male gorilla from Germany to South Africa was facilitated using 75 mg zuclopenthixol and 30 mg haloperidol; this dose resulted in deep sedation, however, making clinical assessment difficult.⁴

NEUROLEPTIC DRUGS TO MODERATE AGGRESSION AND FACILITATE INTRODUCTION IN A MALE GORILLA

A group of adult gorillas had been mixed together in the Gorilla Island complex at Bristol Zoo Gardens (Bristol, U.K.) in 2003. The group consisted of a 27-year-old multiparous female (female 1) who had been at the zoo since 1998, a 21-year-old female (female 2), and a 20-year-old male who arrived together in November 2001. Female 2 had congenital bilateral cataracts, which were removed in 2002, restoring full vision.

In summer 2003, adult males were exchanged with another zoo, and a period of introduction followed. This new male had a history of aggressive behavior resulting in injury to females. This male had been housed in a bachelor group for 9 years after removal from his natal group at 8 years old. He was then moved to another zoo to be with a group of four females. During integration with those females, there were several incidents of aggression, resulting in such severe injury to the females that the zoo stopped further attempts at introduction and offered the male for transfer through the breeding program of the European Endangered Species Program (EEP).

The receiving zoo had a larger gorilla facility and thus was able to maintain the male gorilla in isolation from the females for short periods. On assessment at the new zoo, the male appeared agitated and nervous, as evidenced by excessive sweating, "raspberry blowing" (pursing the lips and blowing), hooting, and exhibiting poor appetite. Despite his previous history, a normal but carefully monitored introduction program was planned to determine if his responses would be better now that he was in a different environment. During the first 7 days the male was in auditory, visual, and olfactory contact but physically separated from the females. Various management practices were attempted to integrate the male with the two females from day 8. The mixes with the male occurred during the day; the females were together overnight, but separated from the male.

On the first introduction the male attacked female 1. This behavior is expected in early gorilla introductions, but this episode was prolonged and severe, and the male appeared to ignore the female's submissive gestures. Female 2 complicated this initial introduction by supporting the male in his attack. Female 2 had no experience of gorilla group dynamics and limited social skills because she recently had congenital cataracts removed, having been virtually blind since

birth. She had also been previously housed in a small zoo with a male gorilla, so there had been limited interaction and no mating between the two animals. During this attack, female 1 sustained a severe injury to the arm necessitating surgical repair. Thereafter, female 2 was not mixed with the male at the same time as female 1 for several weeks in an attempt to integrate the male with one female at a time. Each female was introduced to the male for increasing periods during the day to a maximum of 6 hours per day. Keepers carefully monitored the animals and separated the male when signs of tension were observed. The daily routine involved separating all three animals in the late afternoon for a feed, and then the two females were housed together but separate from the male overnight.

This integration method seemed to be progressing well until day 19, when the male attacked female 1, without provocation, resulting in severe injuries. She had to be separated to permit surgery and healing, although she remained in auditory, olfactory, and visual contact with the male and was housed with the other female at night. The male was therefore mixed only with female 2 for 34 days for 6 hours daily, with only one aggressive incident. On day 23 he attacked her for several minutes but without injury; this was seen as normal behavior. On day 36 there was a mix with both females, and the male gorilla immediately attacked female 1, who sustained severe injuries, again requiring surgery. Given this history of repeated injurious behavior, an attempt to moderate the male's aggression using medication was initiated, because further introductions were deemed to place the females at an unacceptable risk of harm.

Behavioral Management Techniques

The introduction of new males to a captive gorilla group is a potentially dangerous procedure and some fighting may occur, which indeed is normal behavior. Studies on mountain gorillas showed that long-term resident, dominant females received a higher proportion of displays from the dominant males; there was an association between female appeasement reactions and male displays. This suggests that males display to create occasions for the females to confirm their subordination to them. Estrous females did not receive a higher proportion of male displays, and there was no association between male display and copulation.¹⁰

A study of natural behavior in western lowland gorillas found that evidence for an agonistic dominance hierarchy between females is weak; however, rates of

agonistic behavior between females and silverback males were higher. Agonistic relationships between males and females conformed to patterns seen in mountain gorillas.¹¹ Therefore, *excessive* aggressive behavior resulting in severe injury is to be avoided because it is *abnormal* behavior. The natural behavior of the species is that the male will display some aggressive behavior to the females, particularly the dominant female. Female 1 was indeed the dominant female of the two gorillas in the Bristol study, but the male did not respond to her subordinate behavior toward him, and the aggression was so extreme as to be designated "abnormal."

The male's abnormal behavior and attitude were characterized by increased sweating, raspberry blowing, and reduced appetite, suggesting a depressed or fearful attitude and resulting in overaggression, rather than simply being an overly aggressive male. A daily routine was established, and it was quickly found that the male regarded changes to routine as stressful, again as noted by an increase in sweating and raspberry blowing. Therefore, day-to-day routine was kept similar as much as possible during the treatment period. Food items were offered calmly. Eventually the animal's appetite improved, although to normal levels only with the final regimen of sulpiride and haloperidol.

It was also noted that the male gorilla appeared fearful when offered food by keepers. Human movements were slow and calm during interactions with the male. When he was aggressive toward staff by banging the doors or the intervening mesh, no punishment was administered, and the behavior was ignored. This behavior was gradually extinguished during the medication period. The male's agitation increased at the time of estrus in female 2; therefore, initially at these times, female 1 was isolated from the male. Although excitement is often noted when females are in estrus, this does not manifest as aggressive behaviors in captive or wild animals, and therefore such behavior is also abnormal.^{10,11}

Various drug regimens were tried together with behavioral techniques. When addressing the behavior of the overaggressive male gorilla, it was important not to reward the abnormal behavior or to reinforce the male's impression that interactions with females are stressful and fearful events likely to result in punishment. Given the history of repeated and severe attacks on the female, the introductions were managed in the inside accommodation, where techniques could be employed to separate the male quickly if a problem occurred. Although the animals had more room outside, monitoring and intervention would be virtually impossible. Initially, staff had to use aversive tech-

niques to separate the male from the female during his prolonged attacks. This may have inadvertently taught the animal that interactions with the females would always result in a negative outcome. Introductions were therefore finished, wherever possible, before aggressive encounters. The aim was to end each encounter before a fight to allow more positive interactions. It was also important not to "reward" the male for fighting with a female by instantly opening the doors and letting him outside. Instead, if there was an inappropriate aggressive encounter, he was separated from the female(s), then held apart for 10 minutes before being allowed outside. Staff did not punish the male, except in the immediate period of trying to stop an inappropriate attack on a female. Behavioral observations of the gorillas were conducted at different times after the introductions.

Activity and location were recorded at 1-minute intervals using a scan-sampling technique. More general observations were also recorded on an ad hoc basis. This information was used to determine the success of the drug regimen and inform the decisions to increase or lower the doses.

Neuroleptic Therapy

Several neuroleptic regimens were used, as summarized in Table 30-1. All medications were administered orally rather than by injection to prevent increased anxiety from repeated injections and to allow more rapid changes of doses. Initially, diazepam was used for 3 days, then thioridazine and haloperidol for 26 days. Haloperidol was used with a number of other drugs. The aim was to use haloperidol as a "top-up" agent to enhance the neuroleptic effect with minimal increase in sedation. The use of haloperidol with the other drugs was intended to be short term; however, as the case progressed, it became apparent that longer-term therapy would be required. Thioridazine and haloperidol seemed to prevent repeated fighting, but this protocol produced such sedation that the male was not interacting much with the females and therefore not learning from the experience of integration. The more modern drug, risperidone, was used with haloperidol for 11 days and produced minimal sedation, but high doses were required for minimal improvements in behavior. It became apparent that long-term therapy would be required, so another drug was selected in an attempt to use a drug at as low and safe a dose as possible. The final successful regimen of sulpiride (with haloperidol for the first 11 weeks) was continued for 36 weeks.

Table 30-1**Summary of Neuroleptic Use in a Male Gorilla at Bristol Zoo Gardens**

Drug	Dosage (mg)	Duration of Treatment (Days)
Diazepam	100	3
Thioridazine (T) and haloperidol (H)	100-300 (T) 20 (H)	25
Risperidone (R) and haloperidol (H)	6-12 (R) 0-30 (H)	11
Sulpiride (S) and haloperidol (H)	400-800 (S) 60-0 (H)	77 Haloperidol tapered to zero over last 20 days
Sulpiride	800	176 800 mg initially for 75 days, then tapered to zero over last 100 days

Diazepam was used for the first 3 days because the drug was readily available, and if successful, it would have been a simple, short-term solution. Benzodiazepines such as diazepam are used as anxiolytics in behavioral medicine. They can produce physical dependence, however, and thus short courses are preferable. They are also often associated with psychomotor impairment as well as impairment of short-term memory and consequently learning ability.¹ The judicious use of medication in this case was to calm the male and allow him to learn from positive interactions with the females. Any impairment of learning ability, however, would not be useful. Similarly, one major limitation of the use of benzodiazepines in dogs is the risk of disinhibition, which in nervous dogs, for example, may lead to an increased level of aggression.¹ The clinical assessment of this male gorilla was that he was primarily a *nervous* rather than aggressive animal, so the use of diazepam was not likely to lead to a successful outcome. The doses used merely sedated the animal and did not affect his underlying attitude because he still expressed violent behavior; he was slower and therefore less dangerous, however, because the females could easily escape. Diazepam did not prove to be a useful medication to alter his behavior, although it did decrease the anxiety of the females because it prevented the male from injuring them. After 3 days, diazepam was discontinued.

The male gorilla was then dosed with 100 mg of *thioridazine* and 20 mg of *haloperidol*. Thioridazine is a group 2 phenothiazine and is associated with moderate

sedation but has the least extrapyramidal effects of the three groups. Haloperidol is a high-potency butyrophenone neuroleptic. This group has the least sedative, hypotensive, and antimuscarinic effects of the neuroleptics, but these drugs may produce extrapyramidal effects (in humans). Some authorities recommend the use of butyrophenones for aggressive states in dogs.¹ Both thioridazine and haloperidol were given in the evening to maximize the effect for the following morning, when the introductions with the females were attempted. Some mornings the male gorilla was also given 30 to 100 mg of diazepam orally if he seemed agitated before the introduction. After further attacks on the females, the thioridazine was increased to 300 mg. Although this higher dose removed the requirement for diazepam, it resulted in significant sedation. This side effect interfered with the male gorilla's ability to interact positively with the females, and thus it was thought that his behavioral modification was not progressing. Indeed, often when the doors were opened to introduce him to the females, the male merely walked through the doors and lay down to sleep for a while. Another factor to consider was that this drug combination has been associated with sudden death in humans (QT interval prolongation and increased risk of ventricular arrhythmias). If used in humans, careful heart monitoring is recommended. This was not practical in the gorilla, and because the therapy was not producing any positive effects, it was discontinued after 25 days.

The male gorilla was then prescribed 6 mg of *risperidone* with 30 mg of diazepam. Risperidone is a modern neuroleptic with fewer side effects (in humans) than thioridazine. The usual dose range in humans is 4 to 6 mg daily; doses above 10 mg (maximum of 16 mg) are only to be used if the benefits are deemed to outweigh the risks. Some fighting was noted, but this was controlled aggression from the male, and no injuries were inflicted. This was a significant advance. However, each introduction was still characterized by increased agitation in the male, and fighting was frequent. The dose of risperidone was increased to 9 mg, which eliminated the requirement for diazepam. The male was sleeping less than when receiving thioridazine and haloperidol, enabling more positive interactions with the females; however, the maximum integration time was 3 hours. The risperidone dosage was increased to 12 mg plus 20 mg haloperidol, but this resulted in too much sleeping without an extension of the integration time. Although at this point it seemed the original combination of thioridazine and haloperidol had produced a better effect in the male, a return to this therapy was discounted because of the

Table 30-2**Doses and Timing of Sulpiride and Haloperidol, Integration Progress, and Aggressive Behavior in a Male Gorilla at Bristol Zoo Gardens**

Day after first intro	Sulpiride (mg/day)	Haloperidol (mg/day)	Days on stated dose	Group integration	Aggression
75	400	60	13	Mixed with both females for 5 days, then female 2 only for 5 days, then remixed all 3	Attacked female 1 when remixed all 3, so dose changed
87	800	40	3	Mixing all 3 gorillas, 1-2 hr/day only	Mixing all 3 gorillas for 1-2 hr/day, but male persistently aggressive
92	800	60	12	All 3 gorillas mixed 6 hr/day	Attacked female 1, so reduced mixing time
104	800	50	6	All gorillas together 1-3 hr/day, then rest of time, male and female 2 together only, 2 females together overnight	None
110	800	40	5	Male kept with female 2 only during her estrus	None
115	800	50	19	Day 117, start mixing all 3 again, all 3 together all day, male separated out overnight	None
133	800	40	7	All 3 together all day, male separated out at night	None
139	800	30	8	Male kept with female 2 only during her estrus	None
146	800	20	3	All 3 together all day, male separated out at night	None
149	800	10	5	All 3 together all day, male separated out at night	None
153	800	0	75	From day 172, all 3 together all day and night	None
227	400	0	28	All 3 together all day and night	None
255	200	0	48	All 3 together all day and night	None
302	100	0	25	All 3 together all day and night	None
28	0	0	—	All 3 together all day and night	None

risk of potentially fatal side effects. Risperidone treatment was discontinued after 11 days.

Sulpiride was selected as the next neuroleptic agent, to be used initially with haloperidol if required. The haloperidol dose would then be tapered to use sulpiride alone; this regimen is relatively safe for long-term therapy in humans, although its use has not been reported in great apes. Table 30-2 summarizes the sulpiride and haloperidol therapy, with comments on the group dynamics and male aggression. Sulpiride is dosed in humans at 200 to 400 mg twice daily (maximum of 800 mg). The male gorilla was initially given 200 mg sulpiride twice daily with 20 to 60 mg haloperidol. This regimen began on day 75 after initial introductions began. Medication was given in the

morning 2 hours before introduction to the females. The male appeared sedated, but his appetite was much improved, and he began interacting positively with both females. He was occasionally displaying to the females, but not attacking them. The male mated with female 2 on day 83 and female 1 on day 94. The male attacked female 1 again on day 99, resulting in a foot injury that required a toe amputation, so the sulpiride dose was raised to 800 mg (divided between morning and evening doses), with 40 mg haloperidol in the morning. Another short attack occurred later, producing no significant injuries, but the dosage of haloperidol was increased to 60 mg. After a few weeks, haloperidol was lowered to 40 mg, then tapered to zero over 2 weeks (day 152). Agitated behavior (sweating, rasp-



Fig 30-1 Male gorilla receiving neuroleptic (sulpiride and haloperidol) treatment shows normal appearance and posture and lack of perspiration. (See Color Plate 30-1.)

berry blowing) was reduced to almost zero using this combination (Figure 30-1). From day 173 after initial introductions, the group was considered to be calm enough to allow all three animals to be left together all day and all night.

The sulpiride dose was gradually reduced from day 198 to 200 mg twice daily by day 232, to 200 mg once daily from day 255, to 100 mg once daily from day 302, and then all medication was stopped on day 328. Treatment therefore ceased 328 days (46 weeks) after the male's arrival at the zoo, which was 253 days (36 weeks) after the start of the final regimen of sulpiride and haloperidol therapy.

Group Outcome

The male's behavior has changed to a normal pattern that includes mating, socializing, and playing with the females. At this time (3 years after arrival, 21 months after medication stopped) the male is still behaving as a normal gorilla. He chastises the females, when appropriate, but this has not escalated to overaggressive, injurious behavior. His nervous behavior, as evidenced by excessive sweating, poor appetite, and

persistent raspberry blowing, has been obliterated. He has now formed a strong bond with both females, with regular mating. Both females conceived in the first year. Nulliparous female 2 conceived for the first time on day 184, but the pregnancy was lost. Mating with the other female continued. Female 1 later underwent fertility investigation and treatment, which resulted in a conception and miscarriage twice, but no live birth to date. The male was not stimulated to attack during these difficult times and thus did not require medication. A live birth from female 2 occurred 9 months after neuroleptic treatment stopped, 21 months after the initial introduction. The male behaved normally toward the infant and was not separated for the birth.

Female 1 gave birth following fertility treatment 19 months after female 2. The group has remained stable, and the male has required no further treatment.

CONCLUSION

The neuroleptic drug regimen was used to moderate the nervousness and aggressive behavior of the male gorilla. It was beneficial to treat both disorders rather than focus on the violent behavior only. This medication regimen altered the male's behavior, reducing his nervousness and aggressive injurious behavior to normal levels to permit integration of the three gorillas. The gradual reduction in medication permitted the male to adapt to the new environment and social situation without resorting to overaggressive behavior or becoming overanxious.

With the medication now stopped, the male's behavior has remained within normal boundaries when confronted with challenges, such as pregnant females, miscarriages and a live birth, and building noise in and around the gorilla house. Therefore I suggest that this male has now learned appropriate gorilla behavior and is able to cope with changes to his environment without exhibiting nervous signs or manifesting his anxiety as excessive aggression toward the females. This medication regimen, coupled with positive and sensitive management procedures, represents a significant advance in animal welfare because it allowed the integration of a male, previously considered overly aggressive and dangerous with females, into a normal gorilla unit and breeding situation.

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Color Plate 30-1 Male gorilla receiving neuroleptic (sulpiride and haloperidol) treatment shows normal appearance and posture and lack of perspiration. (For text mention, see Chapter 30, p. 249.)

Occupational Exposure to Zoonotic Simian Retroviruses: Health and Safety Implications for Persons Working with Nonhuman Primates

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Nonhuman primates (NHPs) may be naturally infected with a plethora of viruses with zoonotic potential, including retroviruses (Table 31-1). In recent years, concern for the prevalence and zoonotic risk potential of retroviruses in captive NHP collections at zoos has grown. In addition, concern has increased regarding the potential impact of these viruses on captive NHP populations, animal breeding, and transfer of specimens to new zoo collections.

A growing body of ongoing research has documented retroviral disease risks to captive and wild NHP populations, as well as risks of retrovirus transmission to zookeepers, research workers, and other human populations exposed to NHPs by hunting, by keeping primate pets, or after direct contact during visits to Old World countries where NHPs are endemic. Numerous analyses of morbidity, mortality, viral prevalence, and zoonotic risks should be used to develop sound recommendations for good preventive health programs and captive management of NHPs, as well as comprehensive occupational health programs for people exposed to NHPs. Institutions housing NHPs need to review and update their occupational health programs continuously with the latest biosafety and health information associated with retroviral zoonoses to help prevent transmission of these potential pathogens.

Simian viruses present risks to both captive NHP populations and persons exposed to NHPs. This chapter examines the simian retroviral zoonoses that are a concern for the numerous and broad variety of primate taxa that are maintained in zoologic facilities and research institutions. Exogenous simian retroviruses are reviewed as a health concern for zoo and wildlife veterinarians, primate handlers, other persons in direct

contact with NHPs, and other NHPs in captive settings. This chapter addresses health implications for individual animals as well as managed populations in zoos and research institutions, the cross-species transmission and zoonotic disease potential of simian retroviruses, and practices for working safely with NHPs.

SIMIAN RETROVIRUSES

Simian retroviruses, including simian immunodeficiency virus, simian type D retrovirus, simian T-lymphotropic virus, and gibbon ape leukemia virus, have been shown to cause clinical disease in NHPs. In contrast, simian foamy virus, a retrovirus highly prevalent in most NHPs, has not been associated with clinical disease in naturally infected primates. Although it has been shown that human retrovirus infections with human T-lymphotropic virus and human immunodeficiency virus originated through multiple independent introductions of simian retroviruses into human populations that then spread globally, little is known about the frequency and mechanisms of such primary zoonotic events.

Retroviruses are a large and diverse group of enveloped ribonucleic acid (RNA) viruses in the family Retroviridae that replicate in a unique way, using a viral reverse-transcriptase (RT) enzyme to transcribe the RNA genome into linear double-stranded deoxyribonucleic acid (DNA). Retroviruses may be either exogenous in nature, replicating independent of the host genome and transmitted as infectious virions, or endogenous as proviral DNA integrated in the germline of the host and transmitted vertically.

Table 31-1

Simian Retrovirus Infection Documented in Various Nonhuman Primate (NHP) Species*

Genus	Primate Species	Common Name	Retroviruses†
Old World Prosimians			
<i>Otolemur</i>	<i>O. crassicaudatus</i>	Brown greater galago	SFV
Old World Monkeys			
<i>Allenopithecus</i>	<i>A. nigroviridis</i>	Allen's swamp monkey	SFV, STLV, SIV
<i>Chlorocebus</i>	<i>C. pygerythrus</i>	Vervet	SFV, STLV, SIV
	<i>C. sabaeus</i>	African green monkey	SFV, STLV, SIV
	<i>C. aethiops</i>	Grivet	SFV, STLV, SIV
	<i>C. tantalus</i>	Tantalus	STLV, SIV
<i>Erythrocebus</i>	<i>E. patas</i>	Patas monkey	SFV, STLV, SIV‡
<i>Lophocebus</i>	<i>L. albigena</i>	Gray-cheeked mangabey	SFV, SIV
<i>Miopithecus</i>	<i>M. talapoin</i>	Angolan talapoin	SFV, SIV, SRV
	<i>M. ougouensis</i>	Gabon talapoin	STLV, SIV
<i>Cercopithecus</i>	<i>C. albogularis</i>	Sykes's monkey	SFV, STLV, SIV
	<i>C. mitis</i>	Alue monkey	STLV, SIV
	<i>C. lhoesti</i>	L'Hoest's monkey	SFV, SIV
	<i>C. solatus</i>	Sun-tailed monkey	SIV
	<i>C. cephus</i>	Mustached guenon	SFV, STLV, SIV
	<i>C. erythrotis</i>	Red-eared guenon	SIV
	<i>C. ascanius</i>	Red-tailed monkey	SIV
	<i>C. neglectus</i>	De Brazza's monkey	SFV, SIV
	<i>C. mona</i>	Mona monkey	SFV, STLV, SIV
	<i>C. lowei</i>	Lowe's monkey	SIV
	<i>C. campbelli</i>	Campbell's mona	SFV, SIV
	<i>C. denti</i>	Dent's mona	SIV
	<i>C. pogonias</i>	Crested mona	SFV, STLV, SIV
	<i>C. diana</i>	Diana monkey	SFV, SIV
	<i>C. nictitans</i>	Greater spot-nosed monkey	SFV, STLV, SIV
	<i>C. hamlyni</i>	Hamlyn's monkey	SIV
<i>Macaca</i>	<i>M. mulatta</i>	Rhesus macaque	SFV, STLV, SRV
	<i>M. nemestrina</i>	Pig-tailed macaque	SFV, STLV, SRV
	<i>M. fascicularis</i>	Cynomolgus macaque	SFV, STLV, SRV
	<i>M. arctoides</i>	Stump-tailed macaque	SFV, STLV
	<i>M. radiata</i>	Bonnet macaque	SFV, STLV, SRV
	<i>M. fuscata</i>	Japanese macaque	SFV, STLV, SRV
	<i>M. silenus</i>	Lion-tailed macaque	SFV
	<i>M. sylvanus</i>	Barbary macaque	SFV, STLV
	<i>M. tonkeana</i>	Tonkean macaque	STLV, SRV
	<i>M. cyclopsis</i>	Formosan rock macaque	STLV, SRV
	<i>M. nigra</i>	Celebes crested macaque	SFV, STLV
	<i>M. maura</i>	Moor monkey	STLV
	<i>M. nigrescens</i>	Gorontalo macaque	STLV
	<i>M. ochreata</i>	Booted macaque	STLV
<i>Mandrillus</i>	<i>M. sphinx</i>	Mandrill	SFV, STLV, SIV
	<i>M. leucophaeus</i>	Drill	SFV, STLV, SIV
<i>Papio</i>	<i>P. anubis</i>	Olive baboon	SFV, STLV
	<i>P. cynocephalus</i>	Yellow baboon	SFV, STLV, SIV,‡ SRV
	<i>P. papio</i>	Guinea baboon	SFV, STLV
	<i>P. hamadryas</i>	Hamadryas baboon	SFV, STLV
	<i>P. ursinus</i>	Chacma baboon	SFV, STLV, SIV‡

*Primate nomenclature as described by Groves.⁴ Infection determined by presence of cross-reacting antibodies, virus isolation, and retroviral sequences.

†SFV, Simian foamy virus; SIV, simian immunodeficiency virus; STLV, simian T-lymphotropic virus; SRV, type D simian retrovirus; GaLV, gibbon ape leukemia virus; SSV, simian sarcoma virus.

‡Monkey-to-monkey cross-species infection.

Table 31-1—cont'd

Simian Retrovirus Infection Documented in Various Nonhuman Primate (NHP) Species*

Genus	Primate Species	Common Name	Retroviruses†
Old World Monkeys—cont'd			
<i>Theropithecus</i>	<i>T. gelada</i>	Gelada baboon	SFV, STLV
<i>Colobus</i>	<i>C. guereza</i>	Mantled guereza	SFV, SIV
<i>Piliocolobus</i>	<i>P. badius</i>	Western red colobus	STLV, SIV
<i>Procolobus</i>	<i>P. verus</i>	Olive colobus	SIV
<i>Pygathrix</i>	<i>P. nemaeus</i>	Red-shanked douc	SFV
<i>Trachypithecus</i>	<i>T. francoisi</i>	Francois' langur	SFV
	<i>T. obscurus</i>	Spectacled langur	SRV
<i>Semnopithecus</i>	<i>S. entellus</i>	Northern plains gray langur	SRV
Old World Apes			
<i>Hylobates</i>	<i>H. pileatus</i>	Pileated gibbon	SFV
	<i>H. leucogenys</i>	Northern white-cheeked gibbon	SFV
	<i>H. lar</i>	White-handed gibbon	GaLV
	<i>H. syndactylus</i>	Siamang	STLV
<i>Gorilla</i>	<i>G. gorilla gorilla</i>	Western gorilla	SFV, STLV
<i>Pan</i>	<i>P. paniscus</i>	Bonobo, pygmy chimpanzee	SFV, STLV
	<i>P. troglodytes</i>	Chimpanzee	SFV, STLV, SIV
<i>Pongo</i>	<i>P. pygmaeus</i>	Bornean orangutan	SFV, STLV
	<i>P. abelii</i>	Sumatran orangutan	SFV, STLV
New World Primates			
<i>Ateles</i>	<i>Ateles</i> spp.	Spider monkey	SFV
<i>Cebus</i>	<i>Cebus</i> spp.	Capuchin	SFV
<i>Saimiri</i>	<i>S. sciureus</i>	Squirrel monkey	SFV, SRV
<i>Callithrix</i>	<i>C. jacchus</i>	Common marmoset	SFV
<i>Cacajao</i>	<i>Cacajao</i> spp.	Uakari	SFV
<i>Lagothrix</i>	<i>L. lagothrica</i>	Woolly monkey	SSV

All retrovirus genomes are composed of three major genes flanked by long terminal repeats (LTRs). The three major genes include the Gag or group specific antigen, which codes for the viral structural proteins; the polymerase (Pol) gene, which codes for the RT and integrase enzymes; and the envelope (Env) gene, which contains information for the transmembrane and surface proteins of the viral envelope. A smaller genomic region, Pro, is also present in all retroviruses and codes for the protease enzyme used in post-translational processing of viral proteins. Complex retroviruses also contain additional genes coding for regulatory proteins that control viral replication.

Taxonomically, retroviruses are divided into two subfamilies: the Orthoretroviridae, composed of six genera (*Alpharetrovirus*, *Betaretrovirus*, *Gammaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, and *Lentivirus*) and the Spumaretrovirinae, composed of only the *Spumavirus*

(foamy virus) genus. Exogenous retroviruses of simian origin and of veterinary and public health significance are found in five genera in both retrovirus subfamilies, including the type D simian retrovirus (SRV, *Betaretrovirus*), gibbon ape leukemia virus and simian sarcoma virus (GaLV and SSV, respectively; *Gammaretrovirus*), simian and human T-lymphotropic viruses (STLV and HTLV, respectively; *Deltaretrovirus*); simian and human immunodeficiency viruses (SIV and HIV, respectively; *Lentivirus*); and simian foamy virus (SFV, *Spumavirus*). Retroviruses typically cause lifelong, persistent infections, with extended periods of clinical latency before disease development.

Simian retroviruses have received renewed public health interest since it was discovered that HIV types 1 and 2 (HIV-1 and HIV-2) originated zoonotically from cross-species transmission of SIV from infected chimpanzees (*Pan troglodytes*) and sooty mangabeys

(*Cercopithecus atys*) in central and western Africa, respectively.^{1,8,15} Similarly, HTLV type 1 (HTLV-1) has been shown to have originated from cross-species infection with STLV-1 from many primate species, and SFV and SRV infections have been recently observed in persons occupationally exposed to nonhuman primates.^{13,20} Together, these results have heightened awareness regarding the public health significance of these cross-species infections and raised animal and occupational health concerns over the retrovirus status of captive and wild NHPs.

SIMIAN IMMUNODEFICIENCY VIRUS (SIV)

Epizootiology

Simian immunodeficiency virus has been found in more than 30 species of both wild and captive primates.^{1,3,8,15} Seroprevalences as high as 76% have been seen in some naturally infected primates, with higher prevalence found in adults.¹ Strains of SIV from different NHP species may be highly genetically diverse, with reports of 10 distinct phylogenetic lineages that share about 60% genetic identity.^{1,3,8,15} Most SIVs may grow in human peripheral blood mononuclear cells (PBMCs) in vitro, thus providing concern for the zoonotic potential of this virus.¹

Natural transmission of SIV is thought to occur predominantly horizontally through sexual contact or bite wounds and less frequently by vertical transmission. Cross-species transmission of SIV to primates both in the wild and in captivity has been reported.^{1,3} Although New World primates and prosimians are not natural hosts to SIV, and in vitro studies demonstrate resistance of New World monkey cells to SIV infection, the in vivo susceptibility of New World monkeys and prosimians to SIV is unknown.¹⁷

Expression of Clinical Disease

Simian immunodeficiency virus usually produces life-long and clinically inapparent infections in the naturally infected host species. However, when SIV infection jumps from its “natural” host species to a naive species, as was the case of viral transmission between African NHPs (sooty mangabeys) to Asian macaques (genus *Macaca*) in primate vivaria in the 1960s, immunosuppression and disease were demonstrated.¹⁰ Clinical signs of immunosuppression and disease from naturally occurring SIV are rare in African species but have

been recognized in some primates after long-term infections.¹ Asian primates, especially macaques, are not natural hosts of SIV but are very susceptible to potentially devastating acquired immunodeficiency syndrome (AIDS)-like disease when SIV is accidentally or experimentally introduced to a population.^{1,8}

Clinical signs in susceptible populations range from acute epizootic infections to chronically, latently infected animals that may act as disease reservoirs and not show clinical signs for years. Lesions may include nonsuppurative histiocytic meningoencephalitis with syncytial giant cell formation, giant cell interstitial pneumonia, and disseminated giant cell disease. Persistent SIV infection may also result in lymphoproliferative diseases. Similar to HIV infection of humans, SIV infection of macaques has also been reported to cause severe lymphocyte depletion resulting in immunosuppression and the acquisition of a spectrum of opportunistic infections, such as cytomegalovirus, *Candida*, and *Cryptosporidium*, as well as the onset of clinical pathology associated with these agents.¹⁰

Interpretation of Diagnostic Assays

Diagnosis of SIV infection in NHPs and exposed humans is typically made using a combination of serologic and molecular assays. Screening of plasma and sera with enzyme-linked immunosorbent assay (ELISA) tests, using HIV-1 and HIV-2 as antigens, and assays using SIV-specific synthetic peptides has been shown to be useful in detecting cross-reacting antibodies to a variety of SIVs in each of the major phylogenetic lineages. Serologic confirmation of virus infection may be done with Western blot (WB) testing using SIV and HIV-1 or HIV-2 antigens, alone or in combination. Specimens showing WB reactivity to both Env and Gag proteins are considered seropositive. Samples showing reactivity to either Env or Gag alone or in combination with other viral proteins are considered indeterminate and may require additional testing for final resolution. However, caution must be used in interpreting the results if screening for distantly related viral strains, which may only show limited SIV cross-reactivity in these assays, appearing as seroindeterminate or seronegative samples.

Because seroconversion may not be immediate after exposure and infection, new animals or animals with indeterminate serologic results may require testing on arrival and again in 3 to 6 months. During this quarantine period, polymerase chain reaction (PCR) testing for viral sequences with or without virus isolation using PBMCs or other tissues containing lympho-

cytes may also be needed to certify that an animal is SIV negative. Specific PCR primers for the suspected SIV strain may be used for diagnosis and to confirm the serologic results. Generic PCR primers from conserved regions in different SIV genomes may be necessary to confirm infection in cases in which a divergent virus or cross-species infection is suspected. Screening of free ranging NHPs for SIV may be done noninvasively using feces or urine specimens.^{1,3}

Detection of genetically modified SIV/HIV (SHIV) recombinants, typically used as viral inocula in research studies using primates as models of HIV infection, may be complicated by the specific HIV or SIV genes contained in the genetic hybrid. For example, testing for SIV in this case should be restricted to primers located in the SIV portion of the SHIV hybrid. In addition, some SHIVs destroy CD4⁺ T cells so rapidly that an antibody response is not initiated, resulting in false-negative serologic results.¹³ Thus, molecular testing is needed to confirm infection in some SHIV-infected animals and in persons potentially exposed to these viruses.

Human Infection with SIV

As described earlier, cross-species transmission of SIVs from chimpanzees (SIVcpz) and sooty mangabeys (SIVsm) have been linked to the origin of the HIV-1 and HIV-2 epidemics, respectively.^{1,8} Approximately 40 million people worldwide are infected with HIV-1 and HIV-2, with over half in sub-Saharan Africa. Although SIV asymptomatically infects many NHPs present in zoo collections, such as mandrills (*Mandrillus sphinx*), drills (*Mandrillus leucophaeus*), De Brazza's monkeys (*Cercopithecus neglectus*), mangabeys, and talapoin monkeys (*Miopithecus talapoin*), experimental infection of macaques with SIV or the genetically engineered SHIV recombinants may result in a clinical immunodeficiency disease indistinguishable from human AIDS. Therefore, persons working with SIV-infected or SHIV-infected primates have increased risk of exposure to these lentiviruses with unknown health consequences.

To investigate the possible exposure of workers to SIV, a study conducted by the U.S. Centers for Disease Control and Prevention (CDC) tested more than 3000 samples from humans with occupational exposure to NHPs using HIV-2 serologic assays.^{13,20} Two samples (0.06%) were positive for antibodies cross-reactive to SIV, although the sample pool included an unknown number of repeated tests for some participants; therefore the actual prevalence may be slightly higher.

One sample was associated with a laboratory worker previously identified to be infected with SIV who reported handling SIV-infected primate samples and SIV-infected culture material without wearing gloves and while having severe dermatitis of the hands and forearms. The worker remained seropositive for SIV since shortly after the exposure occurred without increases in antibody titer. SIV sequences were detected in this person at two time points surrounding the isolation of SIV (SIVhu) from this individual's PBMCs 2 years after the exposure. The second worker, also identified previously, had remained persistently seropositive for antibodies to HIV-2/SIV for approximately 11 years after a needlestick exposure with SIV-infected macaque blood.¹⁸ A third person with antibodies to SIV had seroreactivity to SIV disappear shortly after a needlestick accident involving an SIV-infected macaque. Evidence of SIV infection in zoo workers has not been reported.^{13,20}

Viral sequences or isolates have not been detected in either the second or the third SIV-exposed person. The viral load in both persons with persistent anti-SIV antibodies is probably low, as evidenced indirectly by the low anti-SIV antibody titers and the difficulty in detecting SIV in their PBMCs. Because high viral loads are associated with disease and transmission in HIV-infected persons, the possibly low viral load in both persistently SIV-infected persons may help explain why they remain free of AIDS-like symptoms.

These results suggest that primary cross-species transmission of lentiviruses may not always result in associated pathology, although additional clinical follow-up of these persons may be necessary to evaluate diseases with periods of long clinical latency. Similarly, "endpoint" infections have been suggested for HIV-2 subtypes C, D, E, F, and G, which, like subtypes A and B, are believed to be the result of cross-species transmission of SIV from sooty mangabeys.¹

TYPE D SIMIAN RETROVIRUS (SRV)

Epizootiology

A simple retrovirus and an oncovirus, SRV may be prevalent up to 90% in some populations of wild and captive macaques and includes five different serotypes (types 1-5).¹⁰ Serotypes 1 and 3 are found mostly in rhesus macaques (*Macaca mulatta*), serotype 2 is found in pig-tailed (*M. nemestrina*) and cynomolgus (*M. fascicularis*) macaques, serotype 4 has only been isolated from a cynomolgus macaque, and serotype 5 was found in rhesus macaques imported from China. Serotype 3

is also known as the Mason-Pfizer monkeyvirus (MPMV).¹⁰ In addition to macaques, SRV has been isolated from squirrel monkeys (*Saimiri sciureus*), spectacled langurs (*Trachypithecus obscurus*), and yellow baboons (*Papio cynocephalus*). All three isolates were determined to be endogenous retroviruses that are found in the germline, thus are present in every host cell, and are recognized as self; therefore, endogenous retroviruses usually do not trigger an immune response and present a seronegative status. A new SRV, designated type 6, has been reported recently in the PBMCs of an Indian langur (*Semnopithecus entellus*), but SRV antibodies and multiple tissues were not tested in this animal to confirm this was also an endogenous retrovirus. Antibodies for SRV have been reported in wild captured talapoin monkeys (*Miopithecus talapoin*), suggesting that this virus may be endemic in primates from West Africa.¹³

Endogenous retroviruses are typically not highly transmissible horizontally. The virus has been isolated from blood, saliva, urine, and other body fluids, and thus SRV is transmitted through sexual contact, bite wounds, and from dam to infant, both transplacentally and postnatally.¹⁰ Latent infections may occur, and apparently healthy carrier animals have been recognized, particularly in cynomolgus macaques (*Macaca fascicularis*). These animals remain clinically asymptomatic but may shed the virus either continuously or intermittently for long periods before simian acquired immunodeficiency syndrome (SAIDS) eventually develops. Asymptomatic virus-positive animals may be antibody negative, making their identification by serology alone difficult.

Expression of Clinical Disease

As the etiologic agent of SAIDS, SRV was associated with outbreaks occurring in the 1980s in many U.S. primate centers. This syndrome has been associated with opportunistic infections, cutaneous and retroperitoneal fibromatosis, necrotizing stomatitis with osteomyelitis (NOMA), acute death, fever, anemia, neutropenia, lymphopenia, thrombocytopenia, hypoproteinemia, persistent diarrhea, lymphadenopathy, splenomegaly, weight loss, thymic atrophy, and fibroproliferative disorders. Disease has been associated only with macaques and may be sporadic in individually housed chronic carrier animals, enzootic in large breeding groups with positive animals, or epizootic with high mortality rates, after virus introduction to a group of naive animals.¹⁰

Interpretation of Diagnostic Assays

Because of these inapparent carrier states and the extremely high mortality rates in some naive macaque populations, adequate testing of both long-term collection animals and newly acquired animals is essential to prevent spread of the virus. Both serologic screening and virologic screening by culture or PCR testing of PBMCs (or both) are needed to detect potentially healthy, virus-positive, but seronegative animals.¹⁰ Seropositive animals that have recovered from clinical disease, but are latently infected and thus negative by viral isolation, may undergo recrudescence and shed virus later.¹¹ Criteria for WB positivity included reactivity to at least one Gag protein (p24, p27) and at least one Env protein (gp20, gp70). Sera showing no reactivity to these antigens are considered negative, whereas sera showing reactivity to a single viral protein are considered seroindeterminate. All nonnegative (i.e., positive and indeterminate) sera are further tested in an indirect immunofluorescence assay (IFA) to provide serologic resolution. It has been suggested that PBMCs may not be the optimal tissue to analyze for detection of latent SRV infections, and that SRV proviral DNA may be more readily detected in bone marrow and other tissues from infected seropositive macaques whose PBMCs are repeatedly virus negative.

Human Infection with SRV

Screening of humans for SRV suggests that these infections are very rare or nonexistent in the general population. Serosurveys have described partial serologic reactivity against SRV in human sera, but additional evidence of infection has been lacking. Antibodies to type D retrovirus have been reported in 2 of 418 persons (0.48%) who were occupationally exposed to macaques at research centers.¹³ One of these workers had persistent, long-standing seropositivity with neutralizing antibody specific to SRV-2, whereas the second person had waning antibody with eventual seroreversion. The inability to isolate virus and the absence of detectable SRV sequences in the PBMCs of these persons suggest low-level viremias. No disease was reported in either individual. The finding of SRV seroreversion in the absence of detectable virus in one initially seropositive individual is similar to the report previously described of an accidental needlestick exposure to SIV in which a transient humoral immune response was documented. These data indicate a possible abortive infection in this person and suggest that

cross-species transmission of simian retroviruses may not always result in the establishment of a persistent infection. Various recently discovered host restriction factors may at least be partially responsible for preventing these infections.¹⁷

Evidence of SRV infection was reported in one patient with AIDS and lymphoma who had no known contact with NHPs.¹³ SRV was isolated from patient lymphoma tissue; bone marrow was positive for integrated proviral DNA for two viral regions by PCR; and antibodies to both Gag and Env SRV viral gene products were detected in the patient's serum by WB analysis and radioimmunoprecipitation assay (RIPA). Genetic characterization of the isolate revealed a close relationship to SRV-3 and SRV-1. This individual had no known history of contact with NHPs or their blood or tissues, and the source of infection remains unknown.

Interestingly, one of the SRV-seropositive participants in the CDC study was also infected with SFV originating from an African green monkey. These results show that working with NHPs may lead to infection with more than one primate retrovirus, providing a biologic environment that could alter the transmissibility and pathogenicity of these viruses.

SIMIAN T-LYMPHOTROPIC VIRUS (STLV)

Epizootiology

Simian T-lymphotropic viruses (STLVs) are complex retroviruses composed of three major groups, termed types 1, 2, and 3. STLV-1 and STLV-2 are antigenically and genetically closely related to HTLV types 1 and 2 (HTLV-1, HTLV-2), respectively.²⁰ STLV has been found in more than 33 species of Old World primates, both in captivity and the wild. The seroprevalence of STLV has been shown to range from 0% to 95% in captive and wild NHPs and increases with age. STLV-1 is found in a variety of Asian and African primates; STLV-2 has been observed only in captive bonobos (*Pan paniscus*); and STLV-3 (previously referred to as STLV-L) has been seen only in African NHPs, such as red-capped and agile mangabeys (*Cercocebus torquatus* and *C. agilis*, respectively), greater spot-nosed monkeys (*Cercopithecus nictitans*), and baboons (*Papio hamadryas*, *P. papio*, and *Theropithecus gelada*). Dual infections with different groups of STLV occur, with STLV-1 and STLV-3 co-infections found in agile mangabeys and baboons. STLV has not been found in New World monkeys in

the wild, although experimental infection of squirrel monkeys and common marmosets (*Callithrix jacchus*) has been described. A single report of an STLV-2-infected spider monkey (*Ateles* sp.) has not been confirmed and is believed to be a laboratory contaminant.¹³ Collectively, STLVs and HTLVs are referred to as *primate T-lymphotropic viruses* (PTLVs).

The close genetic relationship of STLV-1 to HTLV-1 strongly suggests that STLV-1 has crossed over into humans from NHPs. Likewise, the finding of similar STLV-1 genotypes in sympatric primates or captive animals suggests that cross-species transmissions between different primate species may also occur by fighting and in mixed-species exhibits. Transmission of STLV is hypothesized to be by sexual routes because prevalence increases with age. Vertical transmission from dam to offspring may occur, possibly through infected cells in milk.¹³

Expression of Clinical Disease

STLV-1 has been implicated in development of persistent lymphocytosis and abnormal T cells, T-cell lymphomas and leukemia, lymphadenopathy, generalized skin lesions, and splenomegaly in infected individuals.¹³ In three captive lowland gorillas (*Gorilla gorilla gorilla*), STLV was also implicated in a chronic wasting syndrome.¹¹ Interspecies transmission of STLV-1 from macaques to baboons in a primate center resulted in an outbreak of malignant lymphoma in a large number of animals. Clinical presentations included lethargy, low body weights, anemia, pneumonia, skin lesions, and non-Hodgkin's lymphoma.¹³ In contrast, STLV-2 and STLV-3 have not been documented, to date, as being pathogenic in NHPs, but these findings are limited to the identification of only a small number of infected NHPs with no clinical follow-up.

Interpretation of Diagnostic Assays

Screening for STLV infection is performed by using serologic assays such as ELISA or particle agglutination containing HTLV-1 and or HTLV-2 viral lysates, and with IFA by using HTLV-infected cells. Confirmation of infection is done using HTLV-1 WB assays spiked with recombinant Env proteins (GD21) common to both HTLV-1 and HTLV-2 and with peptides specific for HTLV-1 (MTA-1) and HTLV-2 (K55), thus allowing serologic differentiation of HTLV-1 and HTLV-2, respectively. Animals with reactivity to Gag (p24) and

Env (GD21) proteins are considered seropositive, whereas samples showing reactivity to either Env or Gag alone or in combination with other viral proteins are considered indeterminate and may require follow-up testing for resolution. The enzyme immunoassays (EIAs) and WB assays have been shown to be capable of detecting antibodies to a broad range of PTLVs. Interestingly, STLV-3-infected animals have demonstrated a broad pattern of WB cross-reactivity, including indeterminate, HTLV-1-like, and HTLV-2-like.²⁰ PCR testing of PBMC DNA may also be used to determine infection with this virus, and sequence analysis is required for genotyping into the STLV-1, -2, or -3 group.

Human Infection with STLV

As with HIV, evidence suggests that HTLV originated through cross-species infection from STLV-infected NHPs. Since crossing to humans, HTLV has spread globally to at least 22 million persons sexually, from mother-to-child through infected cells present in breast milk, and by exposure to contaminated blood through transfusions and injectable drug use.^{2,20} HTLV-1 causes adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and other inflammatory diseases in about 2% to 5% of those infected.²⁰ HTLV-2 is less pathogenic than HTLV-1 and has been associated with a neurologic disease similar to HAM/TSP.²

The STLV-1-like infections continue to be reported in persons in central Africa exposed to the blood and body fluids of wild NHP populations through hunting, butchering, or keeping of primate pets. The viruses found in these individuals included strains genetically similar to STLV-1 from mandrills, gorillas, common chimpanzee, colobus (*Piliocolobus badius*), and crested mona monkeys (*Cercopithecus pogonias*). In addition to STLV-1-like viruses, a novel HTLV, named HTLV-3 because of its genetic similarity to STLV-3, was recently identified in African hunters. A fourth HTLV, designated HTLV-4, was found in the same population and is most likely of primate origin, although an STLV-4-infected NHP has yet to be identified.²⁰

Despite evidence that STLV may enter into humans zoonotically, screening of sera from 418 persons working with NHPs in zoos and research institutions were all found to be negative for antibodies to HTLV/STLV.^{13,20} These results suggest that the risk for infection with STLV in the workplace may be low. The absence of STLV-1 infection in primate workers may be explained by a lower prevalence of this virus in captive animals because of the inclusion of STLV-1

in pathogen-free breeding programs at many research institutions.

SIMIAN FOAMY VIRUS (SFV)

Epizootiology

Spumaviruses, also known as foamy viruses, have been isolated from many species of mammals, including cats (*Felis catus*), cattle (*Bos taurus*), horses (*Equus caballus*), hamsters (Cricetinae), sheep (*Ovis spp.*), and sea lions (Otariidae). Unlike SIV, STLV, and SRV, which tend to be more geographically and host restrictive, simian foamy viruses (SFVs) tend to be widespread across species and have been identified with high prevalence in many Old and New World monkeys, apes, and prosimians (see Table 31-1). In captivity, more than 70% of adult NHPs are infected with SFV.¹² Less is known about the prevalence of SFV in wild-living primates but rates as high as 62% have been observed in some species.

The wide distribution of SFV among a variety of NHPs has been shown recently to be the result of co-speciation of SFV with the primate host, suggesting a long history of viral evolution and infection in NHPs estimated at more than 30 million years ago.^{13,20} Latent SFV proviral DNA has been found in most cells and tissues of persistently infected animals, with infectious isolates obtained mainly from the oral mucosa and blood. Contact with these two body fluids has been implicated in horizontal transmission of SFV, such as occurs with biting, licking, and transfusions, although sexual transmission is also suspected to occur.¹² More recently, viral RNA was found in the feces of 75% of wild-living chimpanzees, suggesting that contact with feces, especially mucocutaneously, may also increase the risk of SFV infection. Evidence of vertical transmission has been reported in a chimpanzee, although additional data are needed to confirm this route of transmission.¹³ Newborn and infant primates often test negative on losing passive maternal antibodies, but they may acquire positive serologic status from infection when they become juveniles, presumably by contact with infected adults.^{12,13}

Expression of Clinical Disease

Simian foamy virus has a broad host range and may infect many types of cells from a variety of animal species in vitro, including humans, resulting in cytopathology and cell death. Persistent infection of

cell lines with SFV has also been reported. Although SFV infection was reported in one orangutan (*Pongo pygmaeus*) with encephalopathy, no other clinical diseases have been reported with SFV infection in other species of NHP.¹² The pathogenicity of SFV in many species is unclear, and no direct association between infection and disease has been proved. The persistent and subclinical nature of SFV infection may be related to the ancient co-speciation of NHPs with this virus.^{13,20} Although cross-species transfer of SFV has been reported between NHP species, it is unclear if these infections will lead to disease formation in the new host, as occurs with SIV and STLV.¹³

Interpretation of Diagnostic Assays

The SFV genome is organized like other complex simian retroviruses and consists of Gag, Pol, and Env genes flanked by long terminal repeats (LTRs). In WB analysis, seroreactivity in SFV-infected primates is consistently detected to either the p68/71 or p71/74 Gag precursor proteins and is thus considered to be a diagnostic marker of infection in monkeys or apes, respectively. However, the Gag proteins from SFV-infected apes and monkeys share only about 60% amino acid identity and only weakly cross-react in WB assays using a single SFV antigen from either an infected ape or monkey. Therefore, serologic WB testing for SFV antibodies in monkeys and apes, or humans exposed to these primates, requires the use of two tests, one that contains antigen from a monkey and the other containing antigen from an ape, which will allow detection of antibodies to the Old World monkey or ape SFV variants, respectively. Recently, an assay has been designed that combines both ape and monkey SFV antigens into a single WB assay, eliminating the need for two WB tests on each sample.¹³ Other serologic methods (e.g., ELISA, IFA, RIPA) have also been used for the detection of SFV antibody.¹² In addition to serologic testing, PCR testing for SFV sequences in PBMCs, using generic integrase, Pol, and LTR primers, and virus isolation have been used to detect the presence of SFV infection.²⁰ Screening of free-ranging NHPs for SFV using noninvasive collection of urine and feces has also been reported.¹³

Human Infection with SFV

Early studies described a relatively high rate of seroreactivity to SFV among human populations, but these studies lacked definitive evidence of human infection

and were not subsequently confirmed by other investigators using more sensitive tests. Improved diagnostic assays have not documented evidence of foamy virus infection in large numbers of persons in the general population.¹² In contrast, screening of primate handlers and researchers exposed to NHP origin retroviruses revealed that SFV may cross into people with NHP exposure.²⁰ A voluntary study conducted by the CDC screened sera from 418 persons working at North American zoos and primate centers. Fourteen workers (3.35%) were identified as seroreactive to SFV and consisted of both men and women in both facilities in various occupations, including veterinarian, animal handler, and scientist.²⁰ Genetic analysis and serotyping of the SFV found in these persons showed that the infection originated from African green monkeys (AGM) (1 worker), baboons (4 workers), and chimpanzees (9 workers). In a separate study, 4 of 133 persons (3%) who worked with mammals, including NHPs, were found to be seroreactive to SFV in an anonymous serosurvey of 322 zoo workers.¹¹ Antigen-specific WB assays suggested that the SFV infection of these four persons may have originated from apes. Additional studies have identified SFV infection in two additional workers who are infected with either an AGM-like SFV or a chimpanzee-like SFV.¹² SFV screening of 46 exposed Canadian workers identified two seropositive workers (4.3%), including one with a macaque-type SFV infection.¹³

The identification of infection in five workers originating from chimpanzees and baboons, all of whom did not report any specific injuries from either chimpanzees or baboons, although they all worked directly with these NHP species, is important. These results suggest that transmission of SFV to humans from exposure to NHP body fluids may occur more casually than previously thought. These findings reinforce the importance of adhering to appropriate biosafety precautions while working with NHPs, including using personal protective equipment (PPE).

The high prevalence of SFV infection in these workers raises the question of transmission of SFV to persons exposed to NHP in natural settings, such as hunters and persons with primate pets. Recently, SFV infections in persons exposed to NHPs in a natural setting in Africa and Asia have been reported, demonstrating that this virus may be transmitted by hunting, butchering, keeping NHP pets, or visiting religious temples in locations where free-ranging monkeys live.²⁰ SFV infection in these studies was determined by genetic analysis to have originated from mandrills, De Brazza's monkeys, gorillas, and cynomolgus macaques. These results suggest that simian retroviruses

are actively crossing into human populations exposed to NHPs and that humans are susceptible to infection with at least seven different SFV strains.

To help understand the transmissibility of this potentially emerging infectious disease, the spouses of six men identified in the CDC study were tested for SFV infection. Analysis of fresh blood specimens by serologic and molecular assays indicated the absence of SFV infection. Published findings from different studies of SFV-infected humans also suggested that these are asymptomatic infections; however, the limited number of cases, short duration of follow-up, and selection biases inherent in the enrollment of healthy workers all limit the ability to identify either potential disease associations or secondary transmission.^{12,13} Both the absence of transmission of SFV to spouses and the absence of disease in all six workers after 9 to 19 years of infection suggest that cross-species transmission of SFV to humans is not associated with an abrupt change in pathogenicity.¹³

The lack of disease association in the SFV-infected persons is consistent with natural SFV infection of NHP. However, these data may not exclude the possibility of disease occurrence after long latency periods or by transmission via other routes, such as blood donation. A retrospective study of recipients from a blood donor infected with chimpanzee-like SFV failed to identify evidence of SFV infection in two recipients of red cells, one recipient of filtered red cells, and one recipient of platelets.¹³ Nonetheless, more data are needed to better define the risks for SFV transmission through donated blood. Data are also not available to comparatively assess different SFV variants for their relative infectivity, transmissibility, or pathogenic potential in humans. Additional studies are needed to better understand the natural history of SFV infections in humans and to assess the public health implications of these infections.

GIBBON APE LEUKEMIA VIRUS (GaLV)/SIMIAN SARCOMA VIRUS (SSV)

Epizootiology

Gibbon ape leukemia virus (GaLV) is an exogenous, oncogenic, type C retrovirus that has been isolated from the white-handed gibbon (*Hylobates lar*). The virus is shed in urine and feces and may be transmitted horizontally by contact with these biomaterials and is also suspected to be transmitted sexually. Simian sarcoma virus (SSV) has been found in a single

isolate from a fibrosarcoma in a woolly monkey (*Lagothrix lagothrica*) that was housed with a gibbon. SSV has a defective genome that requires the helper virus *simian sarcoma-associated virus* (SSAV) for replication. Genetic analysis shows that the SSV/SSAV complex is similar to GaLV, suggesting that SSV/SSAV is a strain of GaLV acquired through cross-species infection by the woolly monkey.¹³

Expression of Clinical Disease

The presence of the GaLV virus in zoo collections has been associated with lymphoid and myelogenous malignancies, as well as osteoproliferative lesions with marrow infiltration.¹³ SSV/SSAV inoculation of marmosets has produced astrocytomas, fibrosarcomas, and fibromas, although its clinical significance is unknown in captive populations at this time.

Interpretation of Diagnostic Assays

Chronically infected, apparently healthy, antibody-negative but virus-positive gibbons have been reported, making diagnostic screening for GaLV in captive populations difficult. Serologic assays for GaLV and SSV/SSAV are not readily available and have only limited validation. Thus, molecular screening using PCR assays is the preferred method for detection of infection with this group of retroviruses.

Human Infection with GaLV

After the discovery of GaLV in the 1970s, serologic evidence of human infection with GaLV was described in persons with different leukemia hematologic disorders and in sera from healthy humans.¹³ Additional studies could not confirm the previous findings, demonstrating that the observed seroreactivity to GaLV antigens was most likely nonspecific reactivity to cellular antigens contaminating the viral preparations or related antigens present in the fetal calf serum used for cell line maintenance. In addition, testing using more sensitive PCR-based assays has not supported the serologic evidence of GaLV infection. GaLV has been shown to infect many human cell lines in vitro, suggesting that GaLV may also be able to infect humans in vivo.¹³ Because GaLV infection is restricted to essentially one or two primate species, diagnostic tools for NHP and human surveillance are limited. However, given the pathogenicity of this virus in gibbons and woolly

monkeys, public health surveillance for GaLV with improved diagnostic assays may be needed in persons exposed to these primates at work or in the wild.

EPIDEMIOLOGY OF ZOONOTIC SIMIAN RETROVIRUS INFECTIONS OF HUMANS

Nonhuman primates are often used in biomedical research and are typical members of zoo collections and sanctuaries worldwide. Given the ubiquity and high seroprevalence of these retroviruses in their natural hosts, viral exposure to blood and body fluids of NHPs would be expected to occur in persons working directly with captive primates. To evaluate this possibility, the CDC conducted a serologic survey for simian retroviruses in persons exposed to NHPs at North American primate centers, research institutions, and zoos. This voluntary study screened consenting participants for antibodies to SIV, STLV, SRV, and SFV.^{13,20} In addition, specific exposure information and histories of NHP work were obtained with questionnaires completed by the participants.

Analysis of questionnaire data obtained during the first year of this study found frequent exposures to NHP blood, body fluids, and tissues in occupationally exposed workers. The risk for exposure was highest for animal care workers and persons performing invasive procedures and increased with duration of occupational risk. Needlestick or mucocutaneous exposures were reported by 35% of workers with a median of 7.5 years of occupational risk.¹⁸ The laboratory workers and animal care handlers have occupational risk for exposure to simian retroviruses from naturally or experimentally infected NHPs.

Occupational exposure to these retroviruses is a concern not only because of the potential adverse health effects for individual workers who are occasionally infected, but also because transmission in the occupational setting represents a potential route of secondary transmission from infected workers into the general human population.

PREVENTION OF OCCUPATIONAL NONHUMAN PRIMATE ZOONOSES

Because persons exposed to NHPs are at increased risk for infection with NHP zoonoses, institutions employing persons who work with primates should provide comprehensive occupational health and safety plans (OHSPs) for working with NHPs, as well as appro-

priate safety equipment and training to these workers to prevent occupational zoonoses. OHSPs typically include risk assessment and management components to evaluate the risks for both human and animal health and safety, and to determine the appropriate safety equipment, engineering controls, and training required to protect primate workers. Specific recommendations for occupational health services for the prevention and treatment of exposures to primate workers are also included in OHSPs. Excellent guidelines for working safely with NHPs are available in detail elsewhere.^{4,6,14}

Preparation of Bite/Wound Kit for Use in Nonhuman Primate Areas

Primates may be aggressive animals, and bites, scratches, and other cutaneous exposures may occur. Thus, first-aid kits for the treatment of bite wounds and other cutaneous exposures should be easily accessible and readily available to all personnel working with NHPs. As with all medical and first-aid kits, inspection and restocking with kit components should occur regularly, and out-of-date items should be replaced. All staff should be made aware of the kit location and should receive training in the proper first-aid procedures following a primate bite or wound. Box 31-1 lists necessary supplies for a bite/wound kit. All the names, mailing addresses, and emergency telephone numbers of reference laboratories, local physicians, and other

Box 31-1

Contents of Nonhuman Primate Bite Kit

Cleansing/disinfection materials (povidone-iodine or chlorhexidine)
 Sterile surgical scrub brushes
 Sterile basin for soaking large wounds
 Sterile 4 × 4-inch gauze pads for soaking and dressing of wounds
 Sterile saline solution for irrigation of contaminated eyes, nose, or mouth
 Sterile large (60-mL/cc) syringe for saline irrigation of mucosa
 Paper or cloth tape for dressing of wounds
 Sterile examination gloves (various sizes for persons assisting with cleansing and specimen collection)
 Specimen collection and culture materials, including:

- Sterile cotton or Dacron swabs (without metal shafts)
- Sterile vials of viral transport media (check with local human laboratory for preferred media)

A copy of the institutional standard operating procedures and nonhuman primate safety guidelines

health professionals to contact in case of exposure should also be available and preferably posted by a telephone in or closest to the animal work area. A detailed log of all NHP bite wounds or wound exposures should also be maintained at the institution.

EVALUATION AND MANAGEMENT OF A MUCOCUTANEOUS NONHUMAN PRIMATE EXPOSURE

Procedures for management of NHP exposures should be established by a team of infectious disease and occupational health physicians, veterinarians, research personnel, and safety officers at each institution. Following standard first aid guidelines, in the event that a wound or injury is life threatening, the injured person should be transported by ambulance immediately to the nearest health care facility. A copy of the institutional primate bite/wound protocol should be sent to the hospital with the injured person, preferably with an institutional representative who may contact the institutional occupational health provider and veterinarian.

An NHP bite wound or other skin exposure to NHP tissues and body fluids is immediately cleaned by soaking or scrubbing the wound/exposure site with soap or detergent for at least 15 minutes, then rinsing well with water. If eyes or mucous membranes have been exposed, rinse with sterile saline or flowing water for at least 10 minutes. Then, apply a disinfectant such as 0.5% tincture of iodine to the area for 10 minutes. Cover the wound with protective gauze, tape, or bandage. An area supervisor should be contacted as soon as possible to report the injury.

Postcleansing specimen collection (for possible herpes B virus exposure from macaques) should be obtained by swabbing the wound for viral culture after rinsing the wound with water. Contact with the local hospital or clinical pathology laboratory for appropriate media and specific specimen-handling instructions should be done in advance and should be included in the preexposure protocol. Contacting appropriate health services personnel for physician evaluation should proceed in a timely fashion after wound disinfection has begun.

The animal or enclosure/group of animals involved in the exposure should be identified and the institutional veterinarian notified. The veterinarian may then review the animal's or group's medical records and provide relevant information to the occupational health or infectious disease physician. Veterinarians and animal management teams should also determine possible

testing procedures for individual animals or animal groups involved in the exposure as soon as possible.

Postexposure Prophylaxis with Antiretroviral Drugs

Postexposure prophylaxis (PEP) of NHP bite wounds and exposures to NHP body fluids through contaminated needle punctures, scratches, or contact with mucocutaneous junctions should be taken seriously in order to treat these primary exposures and prevent the zoonotic spread of retroviruses that may exist in the animal or specimen. Ideally, a PEP strategy should be developed before an exposure by a team of infectious disease and occupational health physicians and epidemiologists who are familiar with the biology and epidemiology of retroviral zoonoses and the efficacy of available PEP treatments. Existing guidelines for the management of occupational exposure to HBV, HCV, and HIV and recommendations for postexposure prophylaxis may be applicable to other retroviruses also.⁵

Postexposure prophylaxis with antiretroviral drugs may be indicated for some NHP exposures, especially mucocutaneous exposures to body fluids from animals naturally or experimentally infected with SIV or experimentally infected with genetic recombinants of SIV and HIV (SHIV).^{16,19} Similar chemoprophylaxis for SRV and STLV may also be warranted, although the activity of current antiretroviral drugs on these viruses is not fully understood.^{13,16} Because SFV is currently not known to cause disease in either NHPs or accidentally infected humans, PEP with antiretrovirals may not be justified for this virus.

DETERMINATION OF RETROVIRAL STATUS OF NONHUMAN PRIMATE COLLECTIONS

For reasons of both animal health and occupational safety, determination of the retroviral status of NHP collections, as well as that of newly acquired animals, should be considered.¹³ This may be accomplished by initial serial serologic screening of all animals for antibodies to the simian retroviruses discussed in this chapter, followed by additional testing 1 year later to help identify recently exposed animals that may have seroconverted. Serologic testing alone may be sufficient for detection of SIV, STLV, and SFV infection in adult NHPs that are not directly housed with other NHPs with seropositive or unknown infection status. For SRV, initial testing by both serology and virus

detection methods, such as tissue culture or PCR, is required to identify all infected animals.¹⁰ Testing for GaLV is currently not routinely available.

Different laboratories may use different assays and reagents to optimize laboratory tests to detect individual viral variants. Thus, several factors should be considered when choosing a particular laboratory for any test used to determine viral infection, including availability, cost, use of quality assurance measures, and verification of assay validation to document the sensitivity and specificity of the test to particular viral strains of interest. Unusual or unexpected results, particularly in highly endangered species or when breeding groups are being established, may require confirmation in two different laboratories or by different assays. Once an individual NHP has been confirmed to be positive for any retrovirus, it should be considered infected for life, and retesting for that virus is not necessary. If an animal is test negative but housed with positive animals, retesting annually may be necessary to monitor for seroconversion. If all animals in the collection are negative after repeated testing, and no new animals are introduced, alternate-year or every-third-year testing, with serum banking in the years between, is justifiable in some cases, depending on species involved, risk of anesthesia, social housing, and breeding conditions. Even in animals with documented negative retroviral status, however, the potential for spontaneous seroconversion is such that annual testing may be recommended, particularly since seroconversion of one animal may result in subsequent conversion of all cohorts over time.

If possible, negative animals may be isolated from contact with positive animals and screened periodically until a specific agent has been effectively removed from the cohort and institution. The retroviral status of new acquisitions should be determined before their introduction into existing populations. As captive collection size and management allow, positive animals should be introduced only into groups with other positive animals. Introduction of positive animals into known all-negative groups may result in transmission of retrovirus infection and related diseases in the naive animals. The documented differential pathogenicity of some retroviruses between Asian and African species should reinforce the standard practice of preventing direct contact between members of these two groups of NHPs.¹ The pathogenic potential of variants of these viruses among different species of African primates and their ability to infect New World primates and prosimians are largely unknown.

Currently, insufficient information is available for individual risk assessment regarding movement of

NHPs infected with retroviruses to other zoos. The Old World Primate Taxonomic Advisory Group and Species Survival Plan veterinary advisors should be consulted for specific advice (www.AAZV.org).

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CHAPTER 32

Neurologic Disorders in Cheetahs and Snow Leopards

NADIA ROBERT

Worldwide, cheetahs (*Acinonyx jubatus*) in captivity develop a number of health problems rarely observed in free-ranging cheetahs and unusual in other species, especially felids. These include diseases of the central nervous system (CNS) as well as non-CNS diseases. Among the neurologic diseases, cheetah ataxia, caused by a degenerative spinal cord disorder affecting young and adult cheetahs, represents a serious threat to a sustainable captive cheetah population in Europe. Furthermore, several cases of feline spongiform encephalopathy have been diagnosed in European cheetahs. Although the disease has been reported in several large cat species, the relatively high incidence in cheetahs suggests that they may be more susceptible than other zoo felids. In North America, leukoencephalopathy is an emerging neurologic disease of unknown cause and has had a major impact on the Species Survival Plan (SSP) captive breeding program through loss of important founders.

In snow leopards (*Uncia uncia*, formerly *Panthera uncia*), two neurodegenerative diseases characterized by spinal cord white matter degeneration and neuronal chromatolysis, respectively, have been observed in cubs born in European zoologic institutions. Although somewhat similar to the cheetah myelopathy, these disorders appear to occur only sporadically and do not seriously impact the captive breeding population.

This chapter is restricted to the neurologic disorders that have been observed specifically in cheetahs and snow leopards. However, further classic causes of neurologic diseases, such as canine distemper virus infection, tumors, and degenerative spinal diseases involving intervertebral disc diseases and spondylosis, must be considered as possible differential diagnoses, as in any species.

NEUROLOGIC DISEASES IN CHEETAHS

Cheetah Myelopathy

The cheetah myelopathy is a new and unusual neurologic disease characterized by degenerative lesions of the spinal cord and causing ataxia and paresis. It has emerged in the past 20 years in the European Endangered Species Program (EEP) cheetah population and represents a serious threat to a sustainable captive European cheetah population.²⁸ To date, more than 60 cases have been registered in at least 16 different locations in Europe and in Dubai (United Arab Emirates), resulting in the euthanasia of numerous cheetahs that were part of the EEP breeding program. This disease accounts for 25% of all deaths in the European cheetah population and represents a limiting factor in the growth of the European captive population. Cheetahs of every age group are affected, and often several or all cheetahs of the same litter will eventually develop the disease, either simultaneously or successively over several months or years.

The onset of the myelopathy may be peracute, in many cases subsequent to a stressful event (e.g., hand capture of cubs for deworming or vaccination), and is often temporally associated with clinical herpesvirus infection in dams and littermates. The course of the disease is variable, from rapidly progressive ataxia to a slower development that may include stabilization and acute relapsing episodes.

The etiology of the cheetah myelopathy is still unknown, and several causes have been considered, including genetic, environmental, toxic, nutritional (especially copper), and viral factors. Further characterization

of the lesion using molecular biologic techniques, as well analytic and epidemiologic investigations of the environmental status of captive cheetahs (e.g., nutrition, standard medication) are in process and may provide clues to the pathogenesis of this unique disease entity.

Clinical Signs

In cheetah cubs and adults, onset of ataxia or paresis is usually peracute to acute and may occur spontaneously or after a stressful event for the individual or the litter. Events that have been described include hand capture, restraint, and transport for examination or treatment and translocation to a new enclosure. In cubs, clinical signs are often preceded by sneezing and ocular discharge typical of feline herpesvirus type 1 (FHV-1) infection in the dam or littermates.

Whereas clinical onset always starts with pelvic limb ataxia/paresis, disease progression and severity of the symptoms vary considerably among individuals. The clinical neurologic signs indicate an upper-motor-neuron lesion and proprioceptive deficits, with involvement of the long-tract sensory pathways in all cases. After onset of hind limb ataxia, sometimes with involvement of the forelimbs, simultaneous and subsequent recorded symptoms include paresis, staggering, knuckling, swaying high-stepping gait (hypermetria), falling over while turning, dragging of the paws or hind limbs, difficulty rising to a standing position, and finally, in the most severe cases, recumbency. In most cases these clinical symptoms are accompanied by slowly developing wasting (disuse atrophy) of the hind limb muscles. In the standing position the hind legs are typically placed more laterally than normal (abducted) in a base-wide stance, and support of the tail is reduced. In some cases, urinary incontinence was indicated by urine soiling of the perineum. Tremor of the head was observed in some advanced cases.

As previously stated, the course of the disease is variable; the initial ataxia and paresis may develop rapidly to hind limb paralysis and recumbency or may progress slowly and stabilize with mild symptoms for several months or years. Although clinical improvement after tentative treatment was observed in a few cases, relapsing bouts of ataxia or paresis eventually reappeared in most cases. Throughout the disease progression, the affected cheetahs had a normal appetite, did not seem to experience pain, remained alert, and responded to visual and auditory stimuli.^{14,26,27,29}

Epidemiology

To date, more than 60 cases have been recognized in at least 16 different institutions, including zoologic parks

and private owners. The first cases of cheetah ataxia were described in South Africa in 1981,³ but since then, the syndrome has been reported only in Europe and the United Arab Emirates. Some anecdotal evidence from wild-caught cubs in Namibia has been reported.⁹ All affected cheetahs have been captive-bred in a European, Middle Eastern, or South African institutions from captive-borne or wild-caught parents, belonging to the South African subspecies (*Acinonyx jubatus jubatus*) or East African subspecies (*Acinonyx jubatus soemmeringii*). All affected cheetahs were born from parents without prior clinical neurologic signs. Some of the parents were known to have produced other healthy litters before or after the ataxic litters, and individual parents developed ataxia themselves at a later stage. Often, several or all cubs or siblings from a same litter were affected, with symptoms starting simultaneously in all individuals or developing successively over several months or years. There is no apparent gender predilection, and the age of onset of the ataxia ranges from 2.5 months to 12 years.

The captive management and holding conditions vary among institutions that have reported ataxic cheetahs, and no "common denominator" could be identified to date. At most facilities, the cheetahs live in enclosures of varying size with natural soil, usually grassy areas, and heated indoor pens. Ataxia has been recorded at institutions keeping only one pair of animals, as well as institutions holding several cheetahs together or in separated paddocks, usually adjacent to each other. In most institutions the cheetahs are housed in visual or auditory range of unrelated cheetahs or other species. Feeding regimen is mostly composed of a daily meat ration (rabbit, goat, chicken, calf), usually supplemented with a vitamin-mineral additive. In a few institutions the meat is attached to a ski lift-like mechanism that provides a simulated hunting situation, encouraging frequent physical exercise.

Vaccination and deworming of the young and adult cheetahs are routine in all institutions that have reported ataxic animals. A few cubs developed clinical signs before vaccination, but most of the affected cheetahs were routinely vaccinated against feline parvovirus (FPV), FHV-1, and feline coronavirus (FCV) using inactivated or modified live vaccines.^{14,26,29} Some individuals were also vaccinated against feline leukemia virus (FeLV). Known products used for deworming include ivermectin, mebendazole, fenbendazole, febantel, pyrantel pamoate for cubs, pyrantel tartrate, and fipronil.

Clinical Pathology and Ancillary Procedures

Thorough clinical investigations have been carried out in most reported ataxia cases. Although the cheetah

myelopathy has often been temporally associated with clinical herpesvirus infection in cubs, no definitive etiologic factor could be determined.^{14,26,27,29} Plain radiographs, contrast myelography, and magnetic resonance imaging (MRI) were normal. No abnormalities were detected in the cerebrospinal fluid (CSF) or in the urine.

Hematology and blood chemistry values were always within the normal range. Serum copper values (6–22 $\mu\text{mol/L}$) revealed no significant difference between ataxic cheetahs and domestic dogs and cats. Furthermore, there was no significant difference in liver copper levels between ataxic cheetahs (4.6 ± 3 ppm) and cheetahs without CNS disease (4.3 ± 1.5 ppm). However, a significant difference in liver copper has been shown between cheetahs and dogs and cats, but not a wild lynx.²⁹ This difference might be explained by the domestic animals being mostly fed with supplemented commercial food.

Serologic examinations revealed negative or low titers against feline infectious peritonitis (FIP), canine distemper virus (CDV), FPV, FCV, FeLV, feline immunodeficiency virus (FIV), Borna disease virus (BDV), encephalomyocarditis virus, tick-borne encephalitis virus, mucosal disease complex virus, Teschen-Talfan disease virus, *Listeria monocytogenes*, and *Chlamydophila psittaci*. Antibody titers against FHV-1 and *Toxoplasma gondii* were elevated in several cases but negative in another institution, although the cubs had shown ocular discharge and mucopurulent conjunctivitis.¹⁴ The tests for FIP were also negative.²⁶

A herpesvirus was isolated from the eyes and nose of one cub with ocular discharge, and the gene sequence showed 99% overlap with FHV-1.²⁹

At necropsy, ataxic cheetahs are frequently diagnosed with mostly mild or moderate lesions in non-CNS organs. Most of these non-CNS diseases are “classic” diseases frequently observed in captive cheetahs, such as gastritis, enterocolitis, glomerulosclerosis or glomerulonephritis, hepatic or renal amyloidosis, and myelolipoma. However, no correlation could be made with the myelopathy.

Pathology

Gross pathologic lesions in the spinal cord are rarely seen and consist of multifocal, segmental, bilateral, symmetric, grayish white discoloration of the spinal cord white matter.

Histologically, almost exclusively the white matter of the spinal cord is affected in all animals, consisting of continuous columns of white matter degeneration with only occasional presence of chromatolytic neurons in the gray matter. The lesions of the spinocerebellar tracts (laterodorsal funiculi) may extend into the

medulla oblongata up the cerebellar peduncles. Discrete perivascular lymphocytic infiltration may be observed in the brainstem and the spinal cord meninges. In the ventral roots, dorsal roots, and peripheral nerves, rare wallerian degeneration with typical digesting chambers has been noted, as well as occasional chromatolytic neurons in the dorsal root ganglia. Neuronal lipofuscinosis is regularly seen in the brain and spinal cord gray matter in animals older than 6 years. No other lesions are observed in the white and gray matter of the brain.

The pattern, distribution, and severity of histologic lesions vary among individuals. Lesions are most prominent from the distal cervical to midthoracic segments, gradually decreasing in severity toward the craniocaudal direction. The degenerative changes are always bilaterally symmetric and often affect the entire circumferential length of lateral and ventral spinal cord funiculi, involving both ascending and descending tracts. The proper fascicle usually is largely spared, and the dorsal tracts are affected only in a few cases, generally older animals. The degenerative lesions are characterized by ballooning of myelin sheaths, either devoid of axons or containing intact or fragmented axons or macrophages (gitter cells, myelinophages). On the longitudinal sections, intact or slightly swollen axons are often seen within dilated myelin sheaths. Spheroids are observed rarely. Depending on the severity and duration of the lesions, myelin sheath vacuolation is associated with varying degrees of astrogliosis, characterized by gemistocytes and proliferation of fibrous processes. Considering the presence of intact axons within dilated myelin sheaths, the lack of features typical for early axonal degeneration, and the excess of myelin loss compared with axonal degeneration, the white matter lesion has been classified as a primary myelin disorder.^{26,29} However, based on ultrastructural studies, other authors suggest that demyelination must be considered secondary to axonal degeneration.¹⁴

Therapeutic Trials

Because the etiology of the cheetah myelopathy is unknown, no treatment beside supportive care, as appropriate, may be recommended. Numerous treatment attempts have been reported. Products used include the nonsteroidal antiinflammatory drugs (NSAIDs) tolfenamine, flunixin meglumine, and carprofen; the steroids dexamethasone and prednisolone; various supplementary drugs such as vitamin B complex, α -tocopherol, and selenium, a paraimmunity inducer; and serum-neutralizing antibodies against FPV, FHV-1, and FCV.

In summary, it appears that the progression of the disease process was not influenced by drug therapy.^{14,26,27} In few cases, temporary improvement of the ataxia after therapy with acyclovir and prednisolone could be noted, but resurgence of ataxia/paresis reappeared in most cases.²⁹ With oral and intravenous cupric sulfate (CuSO_4) treatment in 4-month-old cubs with ataxia, serum copper could be raised from 2.5–7 $\mu\text{mol/L}$ to 15–70 $\mu\text{mol/L}$, but there was no improvement of the symptoms.²⁷ Similarly, copper supplementation had no effect in the cubs reported in Ireland,¹⁴ and long-lasting, increased dietary copper intake did not prevent the appearance of the disease in several other zoos.

Etiology

Many hypotheses, including genetic, alimentary, toxic-environmental, and infectious factors, have been considered, but to date, no definitive conclusion could be drawn. Investigations to determine the cause of the cheetah myelopathy have been based on known causes of myelopathy in human and domestic animals. Numerous similar, but not identical, human and animal disorders of the spinal cord that feature white matter demyelination have been described, but the etiology is often unknown and the diseases are classified as “idiopathic.” A presumed cause has only been determined in few cases, involving viral, genetic, autoimmune, nutritional-metabolic, toxic, and physical factors. Considering that the cheetah myelopathy has never been reported within the North American, South African, or Japanese populations, and in view of the similar genetic base of these cheetah populations, extrinsic factors, either related to the management or the environment, must be considered. Again, however, no common denominator has been identified to date.

A degenerative myelopathy of presumed inherited basis is known for several dog species, including the Afghan hound, miniature poodle, German shepherd, Siberian husky, Koiker, and Rottweiler.²³ Regarding the cheetah myelopathy, many different founder lines have been affected, suggesting that it is not a familial disease. Additionally, the pattern of incidence does not indicate a genetic basis for this disease. However, a genetic component to general disease predisposition and response cannot be ruled out, and anticipation of multifactorial inheritance might play a role.² In view of the phenotypic similarities of the diseases in the EEP cheetah population with human mitochondrial DNA-associated diseases, the cheetah mitochondrial genome was analyzed to investigate a possible extra-chromosomal genetic basis for the myelopathy. One

heteroplasmic and two homoplasmic single-nucleotide polymorphisms (SNPs) in the mitochondrial complex I of cheetahs with and without neurodegenerative diseases were identified. However, a correlation between these SNPs and the myelopathy could not be demonstrated.⁴

Known nutritional myelopathy entities include swayback and enzootic ataxia in sheep and goats caused by copper deficiency,³¹ equine degenerative myelopathy due to a presumed vitamin E deficiency,²³ degenerative myelopathy related to vitamin B₁₂ deficiency in humans^{8,15} and cat,²⁰ and hound dog ataxia associated with possible methionine deficiency.²¹ As noted earlier, the first cases of cheetah ataxia were described in South Africa in 1981,³ then later in two litters in The Netherlands.³² The disease was ascribed to copper deficiency, based on the copper measurement in the organs and because one cheetah completely recovered after copper supplementation. It is not clear from the description of the cases, however, whether pathologic lesions were similar to the later outbreaks. This copper deficiency hypothesis could not be confirmed by other authors or in my experience. Although a significant difference in liver copper level has been shown between cheetahs and dogs and cats, there was no significant difference in the serum copper level.²⁹ Again, this difference in the liver copper levels could be explained by the domestic animals mainly being fed with supplemented industrial food.

Infectious agents need to be considered as a potential etiology for the myelopathy. Viruses such as CDV or FeLV may cause degenerative lesions in the CNS white matter.^{6,24} However, attempts to identify potential causative infectious agents in the cheetahs have been unsuccessful to date.²⁹ In a recent study, immunohistochemical (IHC) screening for FHV-1, BDV, canine parvovirus (CPV), and CDV antigen of paraffin-embedded and formalin-fixed brain and spinal cord tissues from 25 cheetahs with cheetah myelopathy was performed.²² Despite FHV-1 positivity in serum samples and conjunctival swabs from two litters of cheetah cubs and one positive titer against BDV, as well as the presence of inflammatory lesions in several brain and spinal cord samples, no positive immunolabeling for FHV-1, BDV, CPV, or CDV was demonstrated. Additionally, IHC screening for FeLV antigen was negative, and no cheetah had a positive FeLV titer.

Cheetah Leukoencephalopathy

Leukoencephalopathy is a serious degenerative disease affecting North American cheetahs¹² but has never

been observed in the European (with one exception in the United Kingdom) and South African populations despite thorough investigations. The most distinctive clinical signs are blindness or visual abnormalities, lack of responsiveness to the environment, behavioral change, incoordination, or convulsions. However, some affected cheetahs may have no specific neurologic signs. The disease emerged in 1996, peaked between 1998 and 2001, and is now declining. About 70 animals have been affected to date at about 30 different facilities. Most affected animals are at least 10 years old. The pathologic lesions are restricted to the cerebral cortex and characterized by loss of white matter with associated, bizarre astrocytosis. The cause is unknown, but epidemiologic features suggest exposure to an exogenous agent through diet or medical management. For clinical diagnosis, MRI is the most sensitive method, but confirmation of the disease is based on histopathologic investigations. The cheetah leukoencephalopathy appears to be irreversible, and treatment is limited to supportive therapy.

Feline Spongiform Encephalopathy

Feline spongiform encephalopathy (FSE) affecting domestic and captive feline species is a prion disease considered to be related to bovine spongiform encephalopathy (BSE). FSE has been reported in several nondomestic cat species, including cheetah, puma, ocelot, tiger, lion, and cougar, but the relatively high prevalence in cheetahs suggests that they may be more susceptible than other zoo felids. To date, nine cases of FSE have been diagnosed in cheetahs.^{1,10,11,17,25} All affected cheetahs were older than 5 years, and with the exception of two cheetahs born in France, all were either born in the United Kingdom or imported from there. Clinically, chronic progressive ataxia initially involves the hind limbs but later progresses to involve the forelimbs. Further clinical signs appear with variable frequency and include postural difficulties, hypermetria, muscle tremors (particularly affecting the head), changes in behavior (e.g., increased aggressiveness, anxiety), hyperesthesia and hyperreaction to sounds, ptialism, prominent nictitating membranes, and blindness. The clinical signs usually develop over about 8 weeks. One affected female had a litter when the clinical signs appeared, but she continued to suckle the cubs throughout the disease period until she was humanely euthanized. One of the three cubs later developed the disease at age 6 years. The diagnosis of FSE requires histopathologic examination of the brain and the finding of characteristic vacuolation. It is

broadly accepted that FSE is the result of BSE infection in felids, and the incubation period appears to be 4.5 to 8 years in cheetahs. However, the occurrence of FSE in the offspring of an affected cheetah in France raises the possibility of vertical transmission.

Other Neurologic Disease Observed in Cheetahs

Vitamin A deficiency has been investigated as a cause of a neurologic disease in two adult cheetahs. Pathologically, there was evidence of coning of the cerebellum and ischemic necrosis of the spinal cord.¹³

NEUROLOGIC DISEASE IN SNOW LEOPARDS

Two distinct neurologic disorders have been reported in young snow leopards in France, Switzerland, and Finland. The cause of these diseases remains uncertain. However, several similar diseases in domestic animals have a familial background, and considering the narrow genetic basis of captive snow leopards, a genetic cause is suspected. A preliminary pedigree analysis showed that all affected cubs have common ancestors. However, a pure genetic cause is unlikely because the same ancestors also appear frequently in the lineage of unaffected snow leopards in other institutions.^{7,18,19}

The first spinal cord disorder was diagnosed in a snow leopard litter at a French zoo. At age 3 to 5 weeks, the three cubs of the litter showed clinical neurologic symptoms characterized by head and body tremors and swaying gait, followed by inability to stand and paresis of the hind limbs. Additional clinical findings were loss of body weight and a shaggy hair coat. Because of the progression of the neurologic signs, all three cubs were euthanized at age 9 to 11 weeks and submitted to necropsy. Histopathologic investigations of the nervous system revealed lesions characterized by chromatolytic neurons in the spinal cord, predominantly in the proprioceptive nucleus thoracicus in the proximal lumbar segments. Distinct myelin sheath dilation and axonal degeneration were observed in the corresponding thoracic and cervical ascending spinocerebellar tracts. No changes were seen in the brain, spinal ganglia, and peripheral nerves. The cause of this spinal disorder remains unknown, and no further ancillary procedures were performed. The litter was born from a breeding pair that had previously produced several normal litters.

The second disorder was diagnosed in snow leopard cubs born to three breeding pairs in one Swiss and two French zoologic institutions between 1997 and 2003. This spinal disorder was clinically and pathologically similar to a previously reported neurologic disorder in snow leopard cubs at the Helsinki Zoo in Finland. The disorders appeared in two, three, and respectively, four consecutive litters from each breeding pair, and all cubs born in the affected litters developed neurologic signs. Beginning at age 2 to 4 months, the cubs developed locomotion disorders characterized by swaying gait, hypermetria, and weakness of the hind limbs, associated with progressive muscle atrophy of the hind legs. Further clinical examination was performed, but the interpretation was difficult because the cubs were fearful. However, the spinal patellar and flexor reflexes were present. The only significant abnormalities revealed by ancillary investigations performed on two cubs were a borderline anemia and a low vitamin B₁₂ level in the serum.

Because of the progressive course of the disease, most cubs were euthanized within 1 year of age. Necropsy was performed on five cubs and did not reveal any gross lesions. Histopathologic examination revealed degenerative lesions in all segments of the spinal cord. The lesions were confined to the lateral and ventral columns, with the dorsolateral and ventromedial aspects most severely affected. The changes were characterized by dilation of myelin sheath, containing preserved axons, myelinophages, or axonal debris, associated with astrogliosis and perivascular glial cell cuffs. The loss of myelin was clearly visible in the Luxol-fast blue stain.

The etiology of this second spinal disorder remains unknown, but it seems important to note that the snow leopards in the Swiss zoo and in the two French institutions were fed with chicken only. Two of these institutions changed the diet to a variety of different meats, supplemented with vitamins and trace elements. The cubs born in these zoos after the diet change did not develop neurologic signs, whereas at the third zoo, which continued to feed chicken, the cubs born were again affected. This may be indicative of a vitamin B or other nutritional deficiency as the cause of the degeneration of the spinal cord in the snow leopard cubs in these facilities.

Besides these two degenerative disorders, we have diagnosed a spastic paralysis of the hind limbs in a 4-month-old snow leopard cub. The necropsy revealed a compression of the spinal cord by a mycotic abscess at the level of the fourth lumbar vertebra. Microbiologic culture performed on the abscess material revealed the growth of *Cladophialophora bantiana*. Neurologic diseases resulting from fungal infection are uncommon in

human and domestic cats, but further cases of saprophytic infection involving the CNS have previously been reported in snow leopards. Extramedullary thoracolumbar fungal abscesses, caused by *Scopulariopsis brumptii*, were diagnosed in two young snow leopards,⁵ and an *Aspergillus terreus* meningoencephalitis was reported in a neonatal cub.¹⁶ It has been suggested that snow leopards may be more susceptible to infectious agents present in more temperate climates, because of a relative lack of exposure to infectious organisms in their natural habitat.³⁰

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Nutritional Factors Affecting Semen Quality in Felids

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Proper nutrition is being increasingly recognized as a critical component of captive breeding programs for nondomestic cats.¹ In many cases, especially when commercial feline diets are not available, the lack of reproduction may serve as a sensitive indicator of nutritional deficiencies and provide an early warning for the development of diet-related pathologic conditions. If nutritional problems are not addressed, conservation and breeding programs may fail to achieve their tremendous potential.

There is limited information on the nutrient requirements of most nondomestic felid species. Some digestibility studies have been conducted in small felids, such as the serval (*Leptailurus serval*), lynx (*Lynx lynx*), caracal (*Caracal caracal*), and sand cat (*Felis margarita*),^{6,10} and in large felids, including the tiger (*Panthera tigris*), lion (*Panthera leo*), puma (*Puma concolor*), and leopard (*Panthera pardus*).^{9,29,31} Although diets may be digested differently,¹⁵ the type of diet offered to captive nondomestic felids is based on nutritional requirements for domestic cats (Table 33-1).^{3,19} Until more descriptive research is conducted regarding the nutrient requirements of nondomestic felids, extrapolation from domestic cats is necessary. In North American zoos, this strategy of using the domestic cat as a model for nutrition in the 36 species of nondomestic cats has been effective.

Most felid species maintained in North American zoologic institutions typically are fed a commercial raw meat-based diet (frozen or canned) that has been supplemented and formulated to meet the requirements for domestic cats. Additionally, certain nutrients of specific concern, such as protein, vitamins, and minerals (especially calcium) are formulated into these cat diets. In contrast, some institutions feed raw muscle meat (slab meat) and add a commercial vitamin and mineral supplement formulated for the extensive deficiencies in an all-meat diet (e.g., calcium, fat-soluble vitamins A, D,

and E). Regardless of the primary diet, “whole-prey” carcasses and large bones often are provided as nutritional supplements to maintain healthy teeth and gums, as well as being excellent items for animal enrichment.

PROTEIN AND AMINO ACIDS

The protein requirement of the cat is higher than that of most mammalian species studied.⁷ Generally, a protein requirement is actually a requirement for individual amino acids. The cat’s higher protein requirement may result from a need for more total protein, not only an increased requirement for essential amino acids.²³ In general, proteins from animal matter contain a more balanced amino acid profile and better digestibility than plant proteins. However, the perfectly balanced protein complete in all essential amino acids has not been found for cats. Even the amino acid deficiencies of beef are evident when compared to the nutrient requirements of domestic cats.⁷ Amino acid availability also may be influenced by storage and food processing. Long-term storage may cause degradation of some nutrients. Certain amino acids may be either destroyed or rendered unavailable by the heating that often occurs during canning or extrusion processes.

Two amino acids have a special significance for cats, arginine and taurine. The cat is unusual in its reliance on the amino acid *arginine*. The cat with an arginine deficiency is unable to metabolize nitrogen compounds (through the urea cycle), which produces rapid elevation of blood ammonia levels resulting in ammonia toxicity and death.¹⁸ Other species may require arginine for growth, but in general they do not need it for adult maintenance.

Taurine also is an essential amino acid for cats. The particular importance of taurine in cat nutrition has been studied for more than 20 years. Cats depend on

Table 33-1

Minimum NRC* Nutrient Concentrations Required in Purified Diets for the Growing Domestic Cat Compared with AAFCO† Nutrient Profiles for Growth and Reproduction of Cats Fed Practical Diets

Nutrient	NRC	AAFCO	Minimum‡	Maximum‡	Expected‡
Moisture, %				70	66
Crude protein, %	24	30	30		56
Arginine, %	1.0	1.25			4.8
Histidine, %	0.3	0.31			2.3
Isoleucine, %	0.5	0.52			2.8
Leucine, %	1.2	1.25			4.3
Lysine, %	0.8	1.2			4.3
Methionine + cysteine, %	0.75	1.1			Unknown
Methionine, %	0.4	0.62			3.4
Phenylalanine + tyrosine, %	0.85	0.88			Unknown
Phenylalanine, %	0.4	0.42			1.9
Taurine, %	0.04	0.1-0.2			0.3
Threonine, %	0.7	0.73			2.5
Tryptophan, %	0.15	0.25			0.3
Valine, %	0.6	0.62			2.9
Crude fat, %		9	10	40	20
Linoleic acid, %	0.5	0.5	0.5		Unknown
Arachidonic acid, %	0.02	0.02			Unknown
Crude fiber, %				3	3.0
Acid detergent fiber, %				5	5.0
Ash, %				8	7.8
Calcium, %	0.8	1	0.8	1.6	1.3
Phosphorus, %	0.6	0.8	0.6	1.2	1.2
Magnesium, %	0.04	0.08	0.05	0.09	0.09
Potassium, %	0.4	0.6	0.5		0.5
Sodium, %	0.05	0.2	0.2		0.5
Chloride, %	0.19	0.3			0.3
Iron, ppm	80	80	80		183
Copper, ppm	5	5-15	5		17
Iodine, ppm	0.35	0.35	1		1
Zinc, ppm	50	75	75		110
Manganese, ppm	5	7.5	7.5		20
Selenium, ppm	0.1	0.1	0.1	2	0.5
Vitamin A, IU/kg	3333	9000	10,000		14,000
Vitamin D ₃ , IU/kg	500	750	1000		2400
Vitamin E, IU/kg	30	30	200		470
Vitamin K, IU/kg	0.1	0.1	1		2.5
Thiamin, ppm	5	5	7		15
Riboflavin, ppm	4	4	6		17
Vitamin B ₆ , ppm	4	4	6		28
Niacin, ppm	40	60	60		226
Pantothenic acid, ppm	5	5	10		15
Folacin, ppm	0.8	0.8	0.8		1.0
Biotin, ppm	0.07	0.07	0.1		0.29
Vitamin B ₁₂ , ppm	0.02	0.02	0.03		0.1
Vitamin C, ppm					470
Choline, ppm	2400	2400	2000		2700

*National Research Council: *Nutrient requirements of cats*, Washington, DC, 1986, National Academy Press.

†Association of American Feed Control Officials: Official publication, Atlanta, 1997, Georgia Department of Agriculture, Plant Food, Feed and Grain Division.

‡The minimum or maximum nutrient concentrations allowed in frozen carnivore diets and the expected nutrient concentrations (dry matter basis) are based on the Zoo Diet Analysis database.

taurine for the formation of bile salts and cannot synthesize sufficient taurine to meet their needs. Taurine deficiency is linked with dilated cardiomyopathy and retinal degeneration.^{5,20,21,23} In leopard cats (*Prionailurus bengalensis*), taurine deficiency was observed in males and females fed a commercial canned cat food for 10 to 24 months, resulting in retinal degeneration ranging from focal tapetal lesions to diffuse pigment atrophy and blindness.¹² Composition of the canned diet was modified to prevent dietary deficiencies. Interestingly, taurine deficiency and retinopathy had no influence on male reproductive function in these leopard cats. High concentrations of normal motile spermatozoa were detected each month during the diet-induced taurine deficiency.¹²

In female domestic cats, taurine depletion severely compromises reproductive performance, including an increase in fetal resorption, abortion, and stillbirth.²⁶ Live-born kittens demonstrate numerous neurologic abnormalities, low birth weight, and poor postnatal survival rate caused by inadequate maternal lactation.²⁶ The domestic cat's minimum taurine requirement was studied,^{5,23} and a taurine content of 500 mg/kg dry matter of the diet was adequate for pregnancy in cats.¹⁹ However, when fed commercial canned diets, 2000 mg taurine/kg dry matter was needed.^{8,21} The cause may have been reduced gastrointestinal absorption of taurine or excessive excretion from the digestive tract. New studies suggest that heat treating of cat food may bind the free taurine, making it unavailable to the animal.²⁰

Table 33-2 lists the protein concentrations in "whole-prey" carcasses, with several common commercial diets and various types of muscle meats. An exclusive whole-prey diet consisting of an intact carcass containing bones and viscera is a complete and balanced diet for felids, similar to the diets consumed by wild, free-ranging cats. Although an excellent diet for felids, it is usually expensive and cost-prohibitive to provide sufficient quantities of whole prey for large felids as a daily diet.

FAT

The most concentrated source of energy in the diet is fat, which also gives palatability to foods. Fat provides essential fatty acids and is a carrier of fat-soluble vitamins. The essential fatty acids (linoleic, alpha-linolenic, and arachidonic acids) are involved in many aspects of health, including skin and coat condition, kidney function, and reproduction. Another unusual characteristic of the felid is that essential fatty acid requirements cannot be met solely from linoleic or linolenic acids, as

occurs in most mammals studied. In addition, cats require a long-chain fatty acid, arachidonic acid, which is available only from animal sources.²² This requirement appears to stem from low activity of hepatic desaturase enzymes required to convert linoleic to arachidonic acid.

The crude fat content of most whole prey is higher than the minimum dietary levels for felids. In some species of whole prey (e.g., chicken), neonates have lower body fat concentrations than older prey animals, and skinned, eviscerated carcasses contain lower fat concentrations than the whole bodies of prey animals of the same age.

CAROTENOIDS AND VITAMINS

The functions of many carotenoids remain unknown. Historically, it was thought that the biochemical function of beta-carotene was as a precursor to vitamin A. Recently, the antioxidant function of a few carotenoids, especially beta-carotene, has been revealed. All animals require vitamin A, but certain species may convert some carotenoids to vitamin A. In contrast, the cat requires a preformed dietary source of vitamin A and thus requires a source of animal matter in its diet.

The liver is the major vitamin A storage organ for those species that have been studied. All whole-prey diets (including viscera) analyzed to date would appear to exceed the dietary requirements for cats (~3333 IU/kg dry matter) (see Tables 33-1 and 33-2)¹⁹ without a need for further supplementation. In contrast, all-meat or chicken-neck diets are known to be deficient in essential vitamins, including vitamins A, D, and E.²⁹ Vitamin A deficiencies have reproductive consequences in female felids, primarily pregnancy loss and small litter size.²⁴ Although comparative data are lacking for male felids, vitamins A and E have a pronounced effect on spermatogenesis in other mammals.^{16,17} A 2-month deficiency of vitamin A was reported to cause endocrine changes and complete aspermia in rats.^{13,14} Vitamin E deficiency also influences spermatogenic development in the boar¹⁷ and causes incomplete spermatogenesis and epididymal dysfunction in the rat.⁴

Levels of vitamin A, D, and E in various feline diets are listed in Table 33-2.

MINERALS: CALCIUM AND PHOSPHORUS

The cat's requirements for other nutrients, such as calcium and phosphorus, appear to be similar to those

Table 33-2

Nutrient Composition of Vertebrate Carcasses, Manufactured Diets, and Muscle Meats Compared with the Estimated Nutrient Requirements (Dry Matter Basis) for the Domestic Cat

Diet Source	Dry Matter (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)	Vitamin A (IU/kg)	Vitamin D ¹ (IU/kg)	Vitamin E (IU/kg)
Carcass²							
Mouse, neonate (~4 g)	26.7	50.3	35.5	8.0	ND ³	ND	ND
Mouse, juvenile (~18 g)	29.5	59.2	24.2	10.0	ND	ND	ND
Mouse, adult (~37 g)	33.5	57.4	23.6	11.3	ND	ND	ND
Rat, juvenile (~64 g)	27.7	62.1	20.4	11.3	ND	ND	ND
Rat, adult (~300 g)	34.8	58.6	22.8	10.1	ND	ND	ND
Rabbit, adult (~1900 g)	28.1	63.5	15.3	9.4	ND	ND	ND
Rabbit, eviscerated (~1700 g)	33.5	71.2	14.6	11.1	ND	ND	ND
Chick, 1 day old (~33 g)	21.6	65.8	13.5	8.8	ND	ND	ND
Chicken backs (~340 g)	41.5	21.9	66.7	6.4	ND	ND	ND
Chicken, adult (~1400 g)	40.5	45.0	51.1	6.2	ND	ND	ND
Manufactured Diets							
Feline (Nebraska) ⁴	38.0	47.3	31.6	11.8	10,512	1025	ND
Feline (ZuPreem) ⁵	36.7	43.0	43.0	5.9	ND	ND	ND
Canine (Nebraska) ⁶	31.0	52.0	22.6	8.1	13,125	1250	ND
Carnivore (Dallas Crown) ⁷	40.0	56.0	20.0	7.8	14,000	2400	470
Carnivore (Natural Balance) ⁸	38.0	61.3	22.3	6.5	18,515	ND	403
Muscle Meats⁹							
Horse meat	28.2	71.0	20.9	3.8	— ¹⁰	—	—
Beef	28.7	76.2	21.9	3.6	—	—	—
Pork	27.1	75.6	20.0	3.9	177	—	—
Chicken (dark and light meat)	24.5	87.3	12.6	3.9	578	—	11
Estimated requirements ¹¹	—	>30	—	—	3333	500	30

¹Vitamin D synthesis in the skin of the cat (from 7-dehydrocholesterol) has not been demonstrated.

It is believed that cats must obtain sufficient amounts from the diet.

²Carcass data from United States: Ullrey, Michigan State University, and Allen and Baer Associates.

³ND, No data; component was not analyzed.

⁴Nebraska Brand Feline Diet; raw, horse tissue base (frozen); www.nebraskabrand.com.

⁵ZuPreem Canned Feline Diet; www.zupreem.com. Data from Allen et al, 1996.

⁶Nebraska Brand Canine Diet; raw, horse tissue base (frozen); www.nebraskabrand.com.

⁷Dallas Crown Carnivore Diet 15; raw, horse muscle base (frozen); www.dallascrown.com.

⁸Natural Balance Zoo Carnivore Diet 10; raw, beef muscle base (frozen); www.naturalbalanceinc.com.

⁹Muscle tissue is a poor source of the fat-soluble vitamins A, D, and E. Data from Allen et al, 1996.

¹⁰ "—" Assumed zero.

¹¹Based on minimum nutrient requirements for the growing domestic cat (National Research Council, 1986). There is no specific water, ash, or fat requirement, except that the cat requires the essential fatty acids, linoleic acid and arachidonic acid.

in other mammals. As a food source, a whole-prey vertebrate carcass generally is similar in nutrient composition across species (e.g., rat, mouse, chick, rabbit) and provides adequate amounts of calcium (Ca) and phosphorus (P) and in a satisfactory Ca/P ratio (~1.5:1). In contrast, muscle meat is different in

nutrient composition from whole prey.² Muscle meat is a good source of protein (see Table 33-2), but it is extremely low in calcium, resulting in an inverse Ca/P ratio (Table 33-3).¹ In felids, dietary calcium deficiency causes resorption of bone mineral, greatly reduced bone density, and ultimately metabolic bone disease.²⁸

Table 33-3

Calcium (Ca) and Phosphorus (P) in Vertebrate Carcasses, Manufactured Diets, and Muscle Meats Compared with the Estimated Nutrient Requirements (Dry Matter Basis) for the Domestic Cat

Diet Source	Calcium (%)	Phosphorus (%)	Ca/P Ratio
Carcass¹			
Mouse, neonate (~4 g)	4.0	1.6	2.5:1
Mouse, juvenile (~18 g)	3.8	1.7	2.2:1
Mouse, adult (~37 g)	2.9	1.9	1.5:1
Rat, juvenile (~64 g)	3.1	2.1	1.5:1
Rat, adult (~300 g)	4.8	1.6	3.0:1
Rabbit, adult (~1900 g)	2.4	1.7	1.4:1
Rabbit, eviscerated (~1700 g)	1.9	1.4	1.4:1
Chick, 1 day old (~33 g)	1.8	1.2	1.5:1
Chicken backs (~340 g)	2.2	1.1	2.0:1
Chicken, adult (~1400 g)	1.7	1.3	1.3:1
Manufactured Diets			
Feline (Nebraska) ²	1.6	1.3	1.2:1
Feline (ZuPreem) ³	1.2	0.9	1.3:1
Canine (Nebraska) ⁴	1.9	1.6	1.2:1
Carnivore (Dallas Crown) ⁵	1.3	1.2	1.1:1
Carnivore (Natural Balance) ⁶	1.9	1.3	1.5:1
Muscle Meats⁷			
Horse meat	0.07	0.5	0.14:1
Beef	0.02	0.7	0.03:1
Pork	0.02	0.8	0.03:1
Chicken (dark and light meat)	0.05	0.7	0.07:1
Estimated requirements ⁸	0.8	0.6	1.3:1

¹Carcass data from United States: Ullrey, Michigan State University, and Allen and Baer Associates.

²Nebraska Brand Feline Diet; raw, horse tissue base (frozen); www.nebraskabrand.com.

³ZuPreem Canned Feline Diet; www.zupreem.com. Data from Allen et al, 1996.

⁴Nebraska Brand Canine Diet; raw, horse tissue base (frozen); www.nebraskabrand.com.

⁵Dallas Crown Carnivore Diet 15; raw, horse muscle base (frozen); www.dallascrown.com.

⁶Natural Balance Zoo Carnivore Diet 10; raw, beef muscle base (frozen); www.naturalbalanceinc.com.

⁷Muscle tissue is a poor source of calcium and results in imbalanced Ca/P ratios. Data from Allen et al, 1996.

⁸Based on minimum nutrient requirements for the growing domestic cat (National Research Council, 1986).

Over time, bone demineralization results in fibrous osteodystrophy and nutritional secondary hyperparathyroidism.^{25,28}

The calcium and phosphorus levels of various whole-prey carcasses and several common commercial diets are listed in Table 33-3. For comparison, muscle meats also are listed to illustrate the calcium deficiency and imbalance in calcium and phosphorus.

IMBALANCED DIETS AND FERTILITY IN FELIDS

Latin American Cats

Data from a reproductive survey of felids in Latin America (Mexico, Central America, South America) demonstrate the importance of diet on reproductive health and breeding programs.²⁷ Reproductive

evaluations were conducted on 185 captive, adult male felids representing eight endemic Latin American felid species, including the ocelot (*Leopardus pardalis*), margay (*Leopardus wiedii*), Geoffroy's cat (*Oncifelis geoffroyi*), tigrina (*Leopardus tigrinus*), pampas cat (*Oncifelis colocolo*), jaguarundi (*Herpailurus yaguarondi*), jaguar (*Panthera onca*), and puma, that were maintained under a variety of dietary and other management conditions in 44 zoos or private facilities in 12 Latin American countries. Of the 185 males in the survey, 172 (93%) were wild-born. The remaining 13 individuals were captive-born from wild-born parents. Almost all the small cats (126/129, 98%) were wild-born compared with the larger cats (46/56, 82%).

Diets at 29 of 44 (66%) facilities (representing 128 surveyed males) were considered nutritionally inadequate and were composed almost entirely of red meat (horse or beef) or chicken heads and necks without supplementation with vitamin/mineral mixtures. The remaining 15 (34%) institutions supplemented the diets with whole-prey carcasses, organ meat, or commercial vitamins/minerals. Overall, only 57 of 185 (31%) cats received nutritionally adequate diets.

Of the 185 male cats assessed, the level of successful captive breeding was low, with only 37 males (20%) classified as proven breeders (produced at least one offspring).²⁷ The majority of these proven breeders were jaguars, pumas, and ocelots. Reproductive evaluations of these 185 males revealed that 131 males (71%) had sperm in their ejaculates; however, the mean number of sperm/ejaculate for each species was low compared with counterpart values measured in U.S. institutions. More than half of all ejaculates contained less than 1 million total sperm, which is an extraordinarily low number for felids.^{11,30} Except for the pampas cat, aspermic individuals were observed in all species. Only 87 males (47%) had at least 1×10^6 total sperm/ejaculate, and only 53 males (29%) had 10×10^6 or greater total sperm/ejaculate.

Although multiple management factors (diet, exhibit design, public interaction, general stressors) likely affect reproductive function in male cats, the recurring linkage of poor diets with poor reproduction, under variable management conditions, supports the perception that nutrition is vital for male felid reproductive success in breeding programs.

Semen Quality in Male Felids

Studies conducted in the United States and Southeast Asia further support the importance of nutrition on reproduction, specifically concerning male seminal

traits. Reproductive evaluation of captive pumas in Florida and numerous felid species in Thailand receiving unsupplemented chicken-head or chicken-neck diets revealed males with either a high incidence of oligospermia (low sperm concentration per ejaculate) or aspermia (no sperm in ejaculate) compared with control individuals fed a commercially prepared, balanced feline diet. Interestingly, most of the pumas in Florida appeared to be in good health and generally exhibited normal blood values, as assessed by complete blood count (CBC) and serum chemistry. Most importantly, serum calcium and phosphorus were within normal limits, despite the diets being low in calcium. This is a common finding with imbalanced diets because normal serum calcium is maintained by the continual depletion of calcium from the bones, which may result in metabolic bone disease. The reduced reproductive potential in these cats was only apparent by semen collection and analysis of sperm concentration (sperm density).

Pumas in United States

The most direct evidence of the influence of diet on male reproductive health in nondomestic cats is provided by a dietary study we conducted on pumas held at a private cat facility in Florida (Table 33-4). Six male pumas were maintained solely on chicken-neck diets for periods of at least 10 months before reproductive evaluation. Semen was collected by electroejaculation and assessed for semen quantity (volume, sperm concentration/mL, sperm/ejaculate, motile sperm/ejaculate) and quality (sperm motility, sperm morphology). Puma diets then were changed to a balanced commercial diet (Nebraska Brand Frozen Feline Diet) for at least 6 months, and then cats were reevaluated for the same reproductive traits. No difference existed in body weight or testicular volume between the cats fed an imbalanced chicken-neck diet and the balanced commercial feline diet. With the new balanced diet, sperm motility and sperm morphology were only slightly improved, but the greatest change was in sperm production (Table 33-4). Sperm concentration increased from 2.6×10^6 sperm/mL to 12.0×10^6 sperm/mL of ejaculate. Total sperm per ejaculate also increased from 3.5×10^6 sperm to 32.9×10^6 sperm. Compared with a control puma population (22 males) in North American zoos fed a commercial balanced diet with adequate calcium (Nebraska Brand Frozen Feline Diet), these new values in the six pumas still were relatively depressed, but represented a marked improvement from earlier findings.

Table 33-4

Body Weight, Testicular Volume, and Ejaculate Traits in Pumas Fed a Chicken-Neck Diet before a Commercial Feline Diet Compared with Control Cats in North American Zoos Fed Commercial Feline Diets*

	TYPE OF DIET (DURATION OF DIET)		
	Chicken Necks (>10 months)	Nebraska Brand (>6 months)	Control Pumas
Body weight (kg)	43.9 ±2.8	54.4 ±4.4	53.7 ±1.8
Testicular volume (cm ³)	16.7 ±2.8	20.8 ±3.6	19.3 ±1.0
Ejaculate volume (mL)	1.9 ±0.5	2.8 ±0.6	2.7 ±0.3
Sperm concentration/mL (× 10 ⁶)	2.6 ±2.2 ^a	12.0 ±3.2 ^b	33.4 ±7.9 ^c
Sperm concentration/ejaculate (× 10 ⁶)	3.5 ±2.3 ^a	32.9 ±9.8 ^b	73.2 ±12.8 ^c
Sperm motility (%)	40.0 ±11.7	56.0 ±12.5	53.9 ±4.9
Motile sperm/ejaculate (× 10 ⁶)	2.5 ±2.0 ^a	23.4 ±7.2 ^b	39.3 ±7.8 ^c
Sperm progression [†]	2.6 ±0.1	3.5 ±0.7	3.3 ±0.1
Normal sperm (%)	8.9 ±2.4	20.3 ±10.0	14.0 ±2.3

*Values = mean ±SEM. Six male pumas were fed an imbalanced chicken-neck diet (for at least 10 months) before changing to the balanced commercial Nebraska Brand Feline Diet (for at least 6 months). Control males ($n = 22$) were maintained in North American zoos and fed the balanced commercial Nebraska Brand Feline Diet. Within rows, means with different superscript lowercase letters differ ($p < 0.05$).

[†]Sperm progression is based on a scale of 0 to 5 (5 = best).

Because all other management factors remained constant, these findings provide powerful evidence that nutrient-deficient diets impact directly on male reproduction, especially semen traits and sperm production.

Felids in Thailand

The importance of diet on animal health and reproductive potential also was demonstrated in an international conservation project initiated between zoos in the United States and Thailand. The goal of the project was to develop a comprehensive captive management program for endemic small felids, including fishing cats (*Felis viverrina*), golden cats (*Felis temminckii*), leopard cats (*Felis bengalensis*), and clouded leopards (*Neofelis nebulosa*), maintained in zoologic institutions in Thailand. The project initially focused on assessment of health, reproduction, and nutrition. A total of 54 cats (31 males, 23 females) representing four small felid species in five institutions were evaluated. A physical and dental examination, CBC, serum chemistry, and reproductive evaluation were conducted on each animal. Major health problems were detected, including fractured canine teeth (open root canals), cardiac arrhythmias, heart murmurs, and metabolic bone disease. A high incidence of obesity also was detected, especially in the golden cats (4/7, 57.1%) and

clouded leopards (4/13, 30.8%). The majority of the CBCs were normal, except for elevated white blood cell counts associated with dermatitis or infected root canals. Most serum chemistries were normal, with the exception of compromised renal function in certain cats.

Initial reproductive assessments in 31 males revealed a high incidence of oligospermic ($<10 \times 10^6$ sperm/mL) males and overall reduced total sperm per ejaculates compared with control individuals evaluated in North American zoos. Ejaculates from 12 of the 31 males (38.7%) were oligospermic, which was most severe in the golden cats (4 of 5 males, 80%) and fishing cats (3 of 5 males, 60%) (Table 33-5). Three of 8 leopard cats (37.5%) and only 2 of 13 clouded leopards (15.4%) produced low sperm concentrations. A high incidence of *teratospermia* (abnormal sperm forms) was detected in the fishing cat, golden cat, and clouded leopard, and percentages of abnormal sperm in each species were similar to captive felids in North America. These results were especially noteworthy because the majority of the cats were wild-caught. Nutritional assessment of the diet (chicken heads and necks) revealed an excessive amount of fat and an inadequate amount of protein, essential vitamins, and some minerals. Therefore, nutritional deficiencies were suspected to be the etiology of the bone deformities and the low sperm concentrations.

Recommendations for dietary changes were developed and implemented to include an increase in

Table 33-5

Influence of Nutrition on Sperm Production in Endemic Felid Species in Thailand Zoos Fed an Unsupplemented versus Supplemented Diet Compared with Control Cats in North American Zoos

	Unsupplemented Diet (Chicken Heads/Necks)	Supplemented Diet (Chicken Meat and Vitamins/Minerals)*	Control Cats†
Golden Cat			
Number of males	5	4	19
Number of oligospermic ejaculates‡ (%)	4 (80.0%)	0 (0%)	8 (42.1%)
Sperm concentration ($\times 10^6$ /mL)	7.4 \pm 3.9 ^a	48.0 \pm 22.0 ^b	64.2 \pm 21.2 ^b
Total sperm/ejaculate ($\times 10^6$)	5.4 \pm 3.5 ^a	26.8 \pm 12.0 ^b	21.2 \pm 8.5 ^b
Leopard Cat			
Number of males	8	5	48
Number of oligospermic ejaculates (%)	3 (37.5%)	2 (40.0%)	0 (0%)
Sperm concentration ($\times 10^6$ /mL)	61.6 \pm 12.4 ^a	134.4 \pm 50.4 ^b	153.9 \pm 17.8 ^b
Total sperm/ejaculate ($\times 10^6$)	11.8 \pm 5.4 ^a	31.2 \pm 13.9 ^b	28.7 \pm 5.9 ^b
Fishing Cat			
Number of males	5	9	16
Number of oligospermic ejaculates (%)	3 (60.0%)	1 (11.1%)	2 (12.5%)
Sperm concentration ($\times 10^6$ /mL)	75.7 \pm 34.2	110.8 \pm 29.6	157.2 \pm 58.2
Total sperm/ejaculate ($\times 10^6$)	40.3 \pm 25.4	58.3 \pm 15.7	51.8 \pm 19.1
Clouded Leopard			
Number of males	13	9	132
Number of oligospermic ejaculates (%)	2 (15.4%)	1 (11.1%)	20.0 (15.2%)
Sperm concentration ($\times 10^6$ /mL)	31.4 \pm 5.9	24.0 \pm 5.7	41.2 \pm 4.6
Total sperm/ejaculate ($\times 10^6$)	31.7 \pm 8.9	22.0 \pm 7.7	296 \pm 2.9

Within rows, means with different superscript lowercase letters differ ($p < 0.05$).

*Centrum vitamin/mineral supplement added to 2 kg of eviscerated chicken.

†Control cats were maintained in North American zoos and fed a commercial balanced diet.

‡Oligospermic ejaculates contained a low sperm concentration ($< 10 \times 10^6$ sperm/mL).

protein (using eviscerated chickens with bones) and the addition of a Centrum vitamin/mineral supplement (Wyeth Consumer Healthcare, Madison, NJ). After at least 1 year on the improved diet, reproductive assessments were conducted in 27 males (see Table 33-5). Only 4 of 27 males (14.8%) exhibited low sperm concentrations containing less than 10×10^6 sperm/mL, compared with 38.7% of the males before the improvement in diet. The most striking difference was observed in the golden cats. None of the four male golden cats was oligospermic, in contrast to 80% previously observed on the imbalanced diet (see Table 33-5). Similarly, only one of the nine fishing cats (11.1%) produced an ejaculate with a low number of sperm, compared with 60% before the diet change. A twofold to sixfold increase in sperm production was detected in the golden cats and leopard cats (see Table

33-5). Fishing cats demonstrated a more subtle increase in sperm concentration, and minimum changes were observed in the clouded leopards.

Overall, these data confirm the important link between nutrition, health, and reproduction in a captive breeding program. Because reproduction is the ultimate key to species survival, it is critical that a suitable diet and feeding strategy be developed for international breeding programs of genetically valuable endangered species.

MUSCLE MEAT DIETS

Vertebrates (whole carcasses) are the predominant prey of many mammalian carnivores. The vertebrate carcass generally is similar in nutrient composition

Table 33-6

Rights were not granted to include this table in electronic media.
Please refer to the printed publication.

From Ullrey D, Bernard J: *J Zoo Wildl Med* 20(1):20-25, 1989.

*Combination of 5 g (~1 teaspoon) and 10g (~2 teaspoons), or use of 15 g (~3 teaspoons) is approximately equivalent to 1 tablespoon, although each product should be weighed at least once because of differences in volume/weight relationships.

†Centrum: From A to Zinc; Wyeth Consumer Healthcare, Madison, NJ 07940; www.centrum.com.

across species, at least with respect to major nutrients (see Table 33-2). Protein concentrations are typically quite high. Calcium and phosphorus usually are present in adequate amounts and in a satisfactory ratio (see Table 33-3). In contrast, muscle meats are quite different in composition from whole prey (see Tables 33-2 and 33-3). Although muscle meats typically are good sources of amino acids, some minerals (e.g., sodium, potassium, iron, selenium, zinc) and some B vitamins (e.g., niacin, B₆, B₁₂) are very low, in addition to low calcium (Ca/P ratios ~1:15-1:30), manganese, and fat-soluble vitamins (vitamins A, D, and E).

Use of muscle meats as the sole diet of carnivores once was widespread in North American zoos, with the predictable result: severe and often fatal nutritional bone disease. In both wild and domestic animals, pathologic bone disease resulting from dietary imbalances of calcium, phosphorus, and vitamin D manifests most readily in growing or lactating animals. Although awareness of the problems associated with such diets now is common among zoo staff, slab meat is still often used with problem eaters. Currently, most North American zoos feed commercially prepared, complete carnivore rations to felids (see Tables 33-2 and 33-3). These diets typically are prepared using horsemeat or beef, balanced with vitamin and mineral premixes, and are available as frozen or canned products. The formulations of these diets are quite similar in proximate analysis to whole vertebrate prey, except that the frozen foods lack hide, hair, and hooves. In some zoos, rats, chickens, rabbits, or other vertebrate prey also are offered on a regular or periodic basis in addition to the commercial preparations. It is important that the whole vertebrate prey also are fed a balanced diet.

In contrast, many zoos outside North America use muscle meats extensively because commercial diets are not readily available or too expensive to import. Furthermore, following outbreaks of avian influenza in Asia, many international zoos have switched from poultry diets to beef muscle meat diets for zoo carnivores. When used, these muscle meat diets should be supplemented with vitamins and minerals, particularly calcium.

The feeding of unsupplemented muscle meat also may lead to vitamin A deficiency in some circumstances. Liver may be used as a source of vitamin A; 10 g of beef liver added to 1 kg of muscle meat will provide approximately 15,000 IU vitamin A/kg dry matter. Because the vitamin A content of liver varies greatly, and because other nutrients will be limited in a diet of muscle and liver, a more consistent method of providing appropriate levels of vitamins and minerals has been suggested. Ullrey and Bernard²⁹ propose that appropriately formulated multivitamin/mineral tablets, in addition to steamed bonemeal or a combination of calcium carbonate and dicalcium phosphate, will satisfy nutritional requirements for cats that must be fed muscle meat (Table 33-6).

Supplementing Muscle Meat or Eviscerated-Carcass Diets

Nutritionally (optimally) balanced diets are presented here for supplementing muscle meat or an eviscerated carcass. These diets are based on basic nutritional requirements estimated from the National Research Council (NRC) recommendations for growing domestic cats.¹⁹ Key supplements to the diets presented next

Box 33-1

Nutrient Composition and Directions for a Commercial Vitamin and Mineral Product to Supplement Raw Meat Diets for All Carnivorous Species*

Nutrient Composition of Supplement

Calcium, %	19.2
Zinc, ppm	1200
Manganese, ppm	150
Copper, ppm	160
Iodine, ppm	20
Vitamin K (as menadione), ppm	50
Thiamin, ppm	200
Riboflavin, ppm	200
Niacin, ppm	500
Pantothenic acid, ppm	125
Folic acid, ppm	16
Pyridoxine, ppm	200
Biotin, ppm	5.0
Vitamin A, IU/g	200
Vitamin D ₃ (added), IU/g	40
Vitamin E, IU/kg	8000
Vitamin C, ppm	5000
Taurine, %	5.0

Directions for Use

Mazuri Carnivore Supplement for Slab Meat (#58QC) is designed to be added at 2.0% of wet weight of slab meat (without bone).

Calculations:

1000 g meat \times 0.02 = 20 g supplement/kg meat

For 20 g supplement/kg meat, add 4 teaspoons (5 g each) supplement/kg meat.

*Ingredients in Mazuri Carnivore Supplement for Slab Meat (#58QC) include calcium carbonate, cooked chicken, taurine, menadione dimethyl-pyrimidinol bisulfite (vitamin K), dl-alpha-tocopheryl acetate (vitamin E), l-ascorbyl-2-polyphosphate (vitamin C), zinc sulfate, copper sulfate, nicotinic acid, vitamin A acetate, pyridoxine hydrochloride, riboflavin, thiamine mononitrate, calcium pantothenate, cholecalciferol (vitamin D₃), calcium iodate, folic acid, and biotin.

For locations of Mazuri products (St. Louis, Mo) sold internationally, find dealers on website at www.mazuri.com, or call Mazuri Customer Service Department at 1-800-227-8941.

are a calcium source in sufficient quantity and a multi-vitamin/mineral source. For muscle meat diets, calcium deficits may be alleviated by using calcium carbonate. However, phosphorus concentrations would still be marginal, and Ca/P ratios would be wider than desirable.²⁹ An optimum calcium and phosphorus supplement may be made by using proportions of one-third calcium carbonate (39% calcium) and two-thirds dicalcium phosphate (22% calcium, 18% phosphorus)²⁹ (see Table 33-6). The proportions of calcium and phosphorus in this mixture are similar

to bone. As an alternative, steamed bonemeal (30% calcium, 12% phosphorus) may be substituted for an equal weight of the calcium carbonate–dicalcium phosphate mixture.

Supplemental vitamins and additional minerals may be provided by using Centrum: From A to Zinc tablets (www.centrum.com), a multivitamin/mineral for humans readily available throughout most of North, Central, and South America; Europe; and Asia (see Table 33-6). These tablets weigh about 1.5 g each, and 1 tablet is sufficient supplement for 2 kg of muscle meat (27% dry matter).²⁹ These tablets are scored and may be broken in half for use with 1 kg of muscle meat.

Another option for supplementing muscle meat is a commercial vitamin/mineral premixed supplement containing sufficient levels of calcium (Mazuri Carnivore Supplement for Slab Meat, product #58QC, St. Louis, Mo). The product is available in North America, and information on international dealers in Taiwan, Korea, Japan, and Hong Kong is listed on the website (www.mazuri.com). This product may be added to muscle meat for a balanced diet for carnivores (Box 33-1).

The following diets describe how to supplement all-meat diets or eviscerated whole carcasses for a balanced diet for carnivores.

Muscle Meat Diet (Beef): Diet 1

1. Muscle meat needs to be supplemented with 1 multivitamin/mineral tablet (Centrum: From A to Zinc), 5 g calcium carbonate, and 10 g dicalcium phosphate for every 2 kg of meat.
2. The multivitamin/mineral tablet (Centrum) and the calcium supplements need to be provided in the proper amounts each time muscle meat is fed (see Table 33-6).

Muscle Meat Diet (Beef): Diet 2

1. Muscle meat needs to be supplemented with 1 multivitamin/mineral tablet (Centrum) and 15 g steamed bonemeal (as the source of calcium) for every 2 kg of meat.
2. The multivitamin/mineral tablet (Centrum) and the bonemeal calcium supplement need to be provided in the proper amounts each time muscle meat is fed (see Table 33-6).

Muscle Meat Diet (Beef): Diet 3

Muscle meat may be supplemented with a commercial powder containing both a multivitamin/mineral and a calcium source (Mazuri Carnivore Supplement

for Slab Meat). This supplement should be added at 2% of wet weight of slab meat (without bone). For each 1000 g of muscle meat, a total of 20 g of supplement (~4 teaspoons) is added (see Box 33-1).

Eviscerated Whole Chicken/Rabbit/Rat: Diet 4

1. No vitamin/mineral supplement is required when a whole carcass is fed, including all the viscera (the intestinal tract may be removed).
2. The viscera (including liver, heart, kidneys, spleen) are an important source of vitamin/minerals and must be included in the diet.
3. It is ideal to feed each cat a whole-prey carcass, with body size chosen according to the cat's size. If the carcass must be divided, it should be split lengthwise so that each cat receives half the carcass. Divide the liver, heart, and other viscera or alternate viscera daily between cats.
4. Prey animals must be healthy and maintained on a nutritionally complete feed before their use as food for cats.
5. If the viscera are not available, the eviscerated carcass must be supplemented with the correct amount of vitamin/mineral supplement (1 Centrum tablet for every 2 kg meat). No additional calcium source is needed if the eviscerated carcass contains the bones.

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Baylisascaris Neural Larva Migrants in Zoo Animals

CYNTHIA E. STRINGFIELD

Larva migrans refers to the prolonged migration and persistence of helminth larvae in the organs and tissues of humans and animals, as a normal life cycle occurrence in intermediate hosts; however, it causes extensive tissue damage and inflammation in incidental hosts.³ Disease syndromes caused by the parasite *Baylisascaris* include visceral larva migrans and ocular larva migrans. The disease process seen most often in zoo species, however, involves *neural larva migrans* (NLM), or *cerebrospinal nematodiasis*, and causes fatal or severe neurologic disease. The disease syndrome may vary and is likely dose related and influenced by prior exposure and species differences.^{3,5}

ETIOLOGY AND EPIDEMIOLOGY

Baylisascaris spp. are ascarid nematodes. These species occur primarily in carnivores (*B. procyonis* in raccoons, *B. columnaris* in skunks, *B. melis* in badgers, *B. devosi* in martens and fishers, *B. transfuga* in bears, *B. schroederi* in giant pandas, *B. tasmaniensis* in Tasmanian devils, quolls, and native “cats”) and in rodents (*B. laevis* in marmots and ground squirrels).

Transmission of most *Baylisascaris* spp. involves ingestion of larvae in small mammal intermediate hosts or direct infection by ingestion of eggs. Larva migrans occurs, and the larvae become encapsulated in internal organs and tissues, where they persist for later transmission to the animal, which eats the intermediate host. In addition, ascarid eggs are extremely resistant and long-lived in the environment. Infected raccoons, for example, may shed millions of eggs per day in their feces because one adult female worm produces up to 179,000 eggs per day. Young raccoons become infected by ingesting infective eggs, and older raccoons are infected from third-stage larvae in intermediate hosts (usually rodents). Transmammary and transplacental forms of transmission have not been investigated but are doubtful.³

In human and veterinary literature the primary discussion of NLM focuses on the raccoon roundworm, *B. procyonis*; however, *B. columnaris* of skunks and *B. melis* of badgers also cause this disease. Different species of *Baylisascaris* vary in their central nervous system (CNS) pathogenicity, based on differences in migration and brain invasion, larval aggressiveness, and the host’s ability to wall off the larvae. *Baylisascaris procyonis* and *B. melis* are the most pathogenic, followed by *B. columnaris*.³

Any areas contaminated with feces that contain the previously listed species of *Baylisascaris*, from wild animals living and defecating in the zoo, are sources of infection for susceptible zoo animals. It is important to note that it takes 11 to 14 days, with optimal temperature and moisture (22°–25° C [71.6°–77° F] and 100% humidity), for *B. procyonis* eggs to become infective (second-stage larvae), but under natural conditions it likely takes weeks to months. However, the eggs may remain infective in the environment for years, even surviving harsh winters. Conditions of extreme heat and dryness will kill eggs by desiccation, probably in weeks or months as well.³ Food, bedding, or enclosures contaminated by skunks and raccoons may serve as a source of infection for captive zoo animals. Not only wild (non-zoo collection) animals, but also captive animals purposely housed with susceptible species as a “mixed-species exhibit,” are at risk.

Baylisascaris procyonis is indigenous in raccoons in North America, Europe, and parts of Asia.³ In North America it is more common in the midwestern and northeastern United States and along the West Coast. However, a 2003 report found 22% of raccoons trapped in the metropolitan Atlanta area were positive, discounting prior reports that *Baylisascaris* was not a concern in the South.² Local prevalences of *B. procyonis* may vary, and population density may change over time. Unless active sampling is done, it is impossible to state where this parasite does *not* reside, and the current focus is on screening raccoons, not other

species such as skunks and badgers.⁷ Wild raccoons have been introduced to Asia and Europe as pets, escaping and taking their *B. procyonis* with them.

Additionally, intestinal *B. procyonis* has been recovered from two kinkajous and could be expected to occur in other related procyonids, such as coatimundis and ringtails. It appears that domestic dogs may also serve as adult or intermediate hosts, with infected dogs found in several midwestern states (U.S.) and in Japan.¹

Susceptibility to *Baylisascaris* larva migrans varies among animal groups and species, with a wide-ranging list of more than 90 species; rodents, rabbits, primates, and birds are most often infected. I have also seen numerous cases in Australian marsupials and a case in a fruit bat. Several cases have been seen in carnivores (*Vulpes*, *Canis*, *Taxidea*, *Enhydra*, and *Mustela*). *Baylisascaris* is also thought to naturally occur in opossums.³ No cases have been seen in zoo hoofstock or livestock. Limited or no migration has been seen in sheep, goats, or swine when experimentally infected. Cats and raptors also appear to be resistant to infection. The susceptibility of poikilothermic vertebrates is unknown, but not expected. Prior exposure, concurrent infections, and hormone fluctuations may influence infection. One newborn lamb was diagnosed and only could have been infected prenatally.³

Although raccoon ascarids are the most likely cause of NLM, skunks and less often badgers infected with *Baylisascaris* must be considered. Because it is impossible to differentiate the third-stage larvae of these parasites in histologic sections, species determination in cases is impossible, unless an epidemiologic study is done.³ For example, skunks were a much larger problem with greater abundance at the Los Angeles Zoo, and *B. columnaris* was identified in feces of wild skunks living in the zoo, which was thought to be a greater contributor of disease in that outbreak.⁹

CLINICAL SIGNS

The severity and progression of CNS disease in NLM depends on the number of eggs ingested, the number of larvae entering the brain, the location and extent of migration damage and inflammation in the brain, and the size of the brain. Although experimentally, larvae enter the somatic tissues, eyes, and brain of some species as early as 3 days after infection, clinical signs are usually not apparent until 2 to 4 weeks or later. If migrating larvae leave the brain or become encapsulated and stop migrating, clinical signs may stabilize.³ I have seen this in numerous cases, only to have signs

reappear, probably as a result of reinfection when the animal remains in the same environment. Thus, a “waxing and waning” course may be seen.

Clinical signs are extremely variable and may include depression, lethargy or nervousness, rough hair coat or ruffled feathers, tremors, head and body tilt, circling, jumping, ataxia, leaning, falling, opisthotonos, lateral recumbency, rolling longitudinally, “stargazing,” arching of the head and neck, blindness, nystagmus, motor weakness or posterior paresis, hypertonia or extensor rigidity, paddling movements while recumbent, coma, and death. Arboreal animals may have difficulty with balance and perching, and primates may have difficulty with manual dexterity.³ Intention tremors may be seen and were pronounced in a golden-headed lion tamarin I have seen.⁵ The problem became so severe in this animal that euthanasia was necessary when it became unable to eat.

DIAGNOSIS AND CLINICAL PATHOLOGY

Diagnosis of ascarids in hosts is done as with other species: finding ascarids in the small intestine at necropsy, or passed in the feces, or finding eggs in the feces. Differentiation of *Baylisascaris* spp. is difficult or impossible because of similarities and overlap of morphologic features.³

In zoo patients with NLM, antemortem diagnosis is a disease of exclusion. No specific, commercially available serologic tests exist for animal species. Serologic testing is used in humans but is not commercially available. Eosinophilic pleocytosis of cerebrospinal fluid (CSF), peripheral eosinophilia, and brain lesions on magnetic resonance imaging (MRI) or computed tomography (CT) scan may be seen.³ Most often, however, this disease is diagnosed based on clinical signs and history of exposure, although I have seen NLM in cases in which animal care personnel were emphatic that no exposure could have occurred or seemed highly unlikely. For example, after investigation, a fruit bat was found to have been exposed as a result of dried feces falling through the top of a chain-link fence exhibit into the hanging food container.

Differential diagnosis (depending on species) should include rabies; protozoal disease (toxoplasmosis, sarcocystosis, amebiasis); fungal, bacterial, or viral encephalitis; other larval migration; pesticide toxicoses; trauma; metabolic causes; and neoplasia.³

Histologically, brain lesions consist of focal to diffuse meningoencephalitis, necrosis, and spongiosis caused by eosinophilic and granulomatous inflammation.

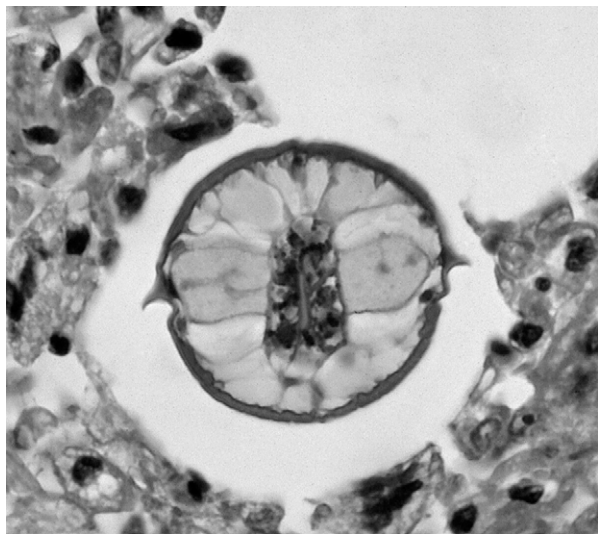


Fig 34-1 Cross section of *Baylisascaris* sp. nematode larva in brain of golden-headed lion tamarin (*Leontopithecus chrysomelas*). Characteristic features include prominent lateral alae and lateral excretory columns that are smaller than the central intestine. (See Color Plate 34-1.) (Courtesy Allan Pessier.)

Grossly, hemorrhagic foci and tracks, congestion, swelling and softening and cerebellar herniation may be seen.

Microscopically, larvae may be seen within the tracks and areas of disease, but may also be found in normal-appearing nervous tissue in the process of causing disease when fixed (Figure 34-1).⁵ Because of their focal distribution, routine histopathology may fail to detect larvae, so numerous blocks and slides must be examined. Larval isolation from the brain of affected hosts may be done by brain squash, artificial digestion, and the Baermann technique. Similar lesions may also be identified in ocular and visceral tissues.

TREATMENT

Definitive hosts may be treated with common anthelmintics at appropriate doses used for ascarids, such as pyrantel; fenbendazole, albendazole, and flubendazole; ivermectin; and moxidectin. Intermediate hosts should only be treated with albendazole and diethylcarbamazine, which cross the blood-brain barrier. However, anthelmintic treatment after CNS disease is pronounced will not be effective in reversing NLM. Killing larvae in the CNS may also increase inflammatory reactions as a result of the release of larval proteins.

Supportive care is indicated, and corticosteroid treatment to decrease inflammation may or may not result in an improvement in clinical signs.

Anthelmintic treatment before larvae migrate to the CNS is preferable. Feeding pyrantel compounds continually in the feed has been used experimentally to stop infection in mice and has been used and recommended in areas where a source of infection cannot be identified or the premises cannot be rid of infection. Periodic treatment with ivermectin has been attempted but has not been as successful as the pyrantel prophylaxis.³ Laser treatment has been successfully used in humans when larvae can be visualized in the retina.

PREVENTION

Baylisascaris infection is a husbandry-related disease and, as such, is completely preventable. Preventing the free-living species that carry this parasite from access to animal exhibits, holding areas, feed, utensils, and related objects will prevent contamination.³ General control measures for these species are similar: preventing access to food, discouraging digging, blocking areas under stairways or porches, pruning trees, and using sheet-metal barriers to prevent climbing.⁸ Skunk dens should be identified and entry/exit sites sealed after observation of the animal leaving the burrow.⁸ Mesh sizes of cages should be small enough to prevent juvenile skunks from entering exhibits.⁹ Any possibly infected feces should be cleaned up before becoming infective.

Mixed-species exhibits should never consist of listed species that are infected with *Baylisascaris* spp. and susceptible species.

In areas open to access by free-living wild animals, a removal or "trap and treat" program must be instituted. In 1995 at the Los Angeles Zoo a trap and humane euthanasia program was instituted because the zoo had become overrun with coyotes, raccoons, and skunks, especially skunks. The county animal control agency was brought in after initial attempts by zoo employees failed, and hundreds of skunks were trapped.⁹

Alternative methods that do not employ euthanasia have been debated. In the past, zoologic institutions have chosen to cordon off contaminated areas and allow wild animals to use them, and zoos have trapped and released animals outside the perimeter fence without testing or identifying them. Neither of these solutions solves the problem, and both are of additional concern in areas that are endemic with rabies. Also, local wildlife regulations may not allow animals to be trapped and relocated. Another approach is to view the animal free-living in the zoo as zoo inhabitants and to develop a trap, treat, and monitoring program,

as reported by the Columbus Zoo.⁴ This program was deemed successful for controlling the raccoon population by creating a healthy population living in the zoo.

Costs, staffing, and other damage or risk of infection by these animals must be carefully considered. Animal rights concerns have also been expressed by the media and public when vermin are controlled by humane euthanasia, creating further considerations.⁹

Dealing with contaminated sites is problematic because of the marked resistance of parasite eggs to all common disinfectants. Small areas of contamination on resistant surfaces may be treated with a 1:1 mixture of xylene and ethanol after organic matter has been removed. Treatment with 20% bleach (1% sodium hypochlorite) will remove the outer protein coat, making the eggs nonadherent and able to be washed away, but it will not kill them. Heat (boiling water, autoclave, steam cleaner) is the recommended method to kill eggs, with direct flame from a propane gun being the most efficient method.

Surface soil may be flamed, broken up, and turned over several times with a shovel or rake, re-flaming each time to ensure decontamination. For heavily contaminated areas or areas where heat is not appropriate, removing and discarding the top several inches of soil is recommended.

Centrifugal sedimentation/flotation on detergent-washed samples may be used to assess the presence of eggs in soil or environmental debris and to evaluate the effectiveness of the chosen technique. Infected disposable items should be incinerated or properly disposed of otherwise.³

ZOONOTIC POTENTIAL

Baylisascaris procyonis is considered to be an emerging helminthic zoonosis.⁷ It principally affects young children but has infected adults as well. It typically results in fatal disease or severe sequelae, as in other animals.

The first recognized human case was reported in 1984 in a 10-month-old child in Pennsylvania. Since then, at least 11 additional cases of severe or fatal encephalitis have been identified.⁶ It is thought that human baylisascariasis is probably underrecognized, and the full spectrum of clinical disease is unclear because the agent is unknown to most clinicians and typically not included in a differential diagnosis. The disease is also difficult to diagnose.⁷

The prevalence of asymptomatic infection in human populations has yet to be determined. Strong recommendations regarding public health have been made

to educate the medical community and the public and to develop better testing. Further study of the infection in U.S. raccoon populations has also been recommended because raccoons readily adapt to human habitation, and population numbers may be increasing in urban areas. The prevalence of asymptomatic infection in human populations is still to be determined.⁷

Zoo personnel are also at risk because of the zoonotic nature of this parasite. Personnel contacting and cleaning contaminated areas should wear disposable coveralls, rubber gloves, washable rubber boots, and a particulate face mask to prevent the inhalation or ingestion of any eggs stirred up in dust.³ All should be educated about the risk of infection.

EDUCATION

Of paramount importance is education of zoo husbandry staff about this parasite. Animal personnel are often sympathetic to feeding or "ignoring" free-living raccoons and skunks and reluctant to trap when they know the animal will be euthanized. Routine reminders and ongoing education are necessary to change this mindset. If personnel are involved in the extensive cleanup efforts, see their animals die or suffer permanent neurologic impairment, and realize they too are at risk of such infection, their sympathy is often changed to support efforts to eliminate the species responsible for such risk from the zoo population.

Constant vigilance and education must be maintained; it is easy to forget and allow exhibits to become overgrown, fence holes to develop, and trashcan lids to fit improperly. As standard prevention, animal care personnel should be educated about essential sanitation habits, such as effective handwashing and avoiding hand-to-mouth contact while working.

CONCLUSION

Baylisascaris infection in zoo animals probably is much more common than currently recognized, and it is diagnosed only sporadically on necropsy in most zoos using a common, private zoologic pathology service (personal communication, Michael Garner, Northwest Zoopath). "Naturalistic" exhibits may pose a challenge because vermin feces may be difficult to see in lushly planted exhibits.

When clinical signs suggestive of this parasite are seen, thorough brain evaluation should be attempted at necropsy. At the Los Angeles Zoo, necropsy reports would often indicate suspicion, but not until further

evaluation was urged and conducted would the parasite be found.

Animals suspected of having this disease should be immediately removed from their environment to prevent the spread of infection. Caregivers should be reassured that the animal is not a risk to them, but that they are at risk if the animal is an infected skunk or raccoon.

Environments must be thoroughly evaluated to discover the route of contamination so that it may be decontaminated. Most importantly, contaminated skunks and raccoons must be excluded from the environment of the captive animals we are entrusted to care for and protect.

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Color Plate 34-1 Cross section of *Baylisascaris* sp. nematode larva in brain of golden-headed lion tamarin (*Leontopithecus chrysomelas*). Characteristic features include prominent lateral alae and lateral excretory columns that are smaller than the central intestine. (For text mention, see Chapter 34, p. 286.) (Courtesy Allan Pessier.)

CHAPTER 35

Use of Analgesics in Exotic Felids

EDWARD C. RAMSAY

Treatment of pain in domestic and nondomestic cats has been a challenge for the clinician. Many cat species are stoic and show few or subtle external signs of pain. Additionally, the adverse effects of nonsteroidal antiinflammatory drugs (NSAIDs) in domestic cats are well documented and have discouraged many practitioners from trying novel NSAIDs in exotic felids.

As in other animals, each cat's response to pain and analgesics will vary, necessitating an individualized treatment plan. As a rule, always treat painful felids to effect, not by rote reliance on published dosages. It is frequently necessary to try different agents and combinations to find which produces the optimal analgesic effect in exotic felids. To minimize adverse effects, work toward treatment with the lowest effective dose when treating chronic pain.

NONSTEROIDAL ANTIINFLAMMATORY DRUGS

The NSAIDs act both centrally and peripherally. The primary effects are believed to be caused by the ability of NSAIDs to inhibit cyclooxygenase (COX) enzymes in the arachidonic acid metabolism cascade. The COX-1 isoform is regarded as constitutive (continuously expressed) and is responsible for many homeostatic processes, such as maintenance of gastric mucosal integrity, platelet function, and renal autoregulation. Reduced COX-1 activity is believed to be responsible for many of these agents' adverse effects. The COX-2 isoform is generally thought to be induced in response to noxious stimuli and initiates creation of proinflammatory prostaglandins. Inhibition of the COX-2 isoforms prevents the production of these prostaglandins and the associated pain. This simplification of COX and NSAID physiology is under constant revision but remains the basis for categorizing the activities of

NSAIDs. All drug dosages, unless otherwise indicated, are as listed in Table 35-1.

Aspirin is the only NSAID for which a safe chronic dosage has been established in cats, but it has been largely replaced in practice by newer NSAIDs. Aspirin has antiinflammatory, antiplatelet, and analgesic effects but is not effective for severe pain. Cats lack the ability to bind many compounds to glucuronides and do not metabolize and excrete salicylates well. As a result, aspirin must be given at a less frequent dosing interval in cats than in most other mammals. Vomiting and gastric hemorrhage are the two major adverse effects seen with aspirin administration.

Ketoprofen and carprofen are propionic acid derivatives with analgesic and antiinflammatory properties. *Ketoprofen* is approved for use in cats in Europe and Canada, in both injectable and oral preparations. Ketoprofen is considered a COX-1 preferential agent and provides good postoperative analgesia in cats, but it reduces platelet activity and therefore is not recommended for administration before surgery. Short-term treatment (up to 5 days) has been investigated in domestic cats, but longer-term therapy has not been studied.

Carprofen is not approved for use in cats in the United States but is approved as a single-dose agent in Europe. It is considered a COX-2 preferential agent and has been studied as a perioperative analgesic for cats. Several authors caution against long-term use of carprofen in cats,¹¹ and there is an anecdotal report of a leopard dying with intestinal ulcers after 2 weeks of carprofen therapy.

Meloxicam is an enolic acid compound with analgesic, antiinflammatory, and antipyretic activities, and it is considered a COX-2 preferential NSAID. It is approved for use in cats in Europe and the United States. The injectable form is approved for single-dose administration, and the oral form is approved for administration up to 5 days. Both the oral and the

Table 35-1

Analgesics of Potential Use in Nondomestic Felids

Agent	Perioperative Use	Short-Term Use	Long-Term Use
Buprenorphine	0.01-0.02 mg/kg SC; may also given IM or PO bid-qid	—	—
Butorphanol*	0.1-0.4 mg/kg SC	0.4-1.0 mg/kg PO q4-8h	0.09-0.2 PO bid
Tramadol†	—	1-4 mg/kg PO bid	—
Aspirin	—	—	10 mg/kg PO q72h
Ketoprofen	2 mg/kg SC	1.0 mg/kg PO q24h	—
Carprofen	4 mg/kg IV, IM, or SC	2.0-2.2 mg/kg IM/SC/PO bid for 2 days	—
Meloxicam	0.2-0.3 mg/kg SC	Day 1: 0.1-0.2 mg/kg PO once Days 2-4: 0.05-0.1 mg/kg q24h Days 5 on: 0.025 mg/kg q48h	—
Piroxicam	—	0.3 mg/kg PO q24h for 4 days, then q48h	—

*4-5 mg SC (total dose for adult tiger).

†50-100 mg (total dose per adult lion or tiger) PO bid.

SC, Subcutaneously; IM, intramuscularly; IV, intravenously; PO, orally; bid, twice daily; qid, four times daily; q4-8h, every 4 to 8 hours.

injectable forms of meloxicam have been used for extended periods in domestic and nondomestic felids with few, if any, adverse effects.

Piroxicam is not related structurally to other NSAIDs but is considered a COX-1 preferential agent. It has antiinflammatory and analgesic properties but has been used most frequently in domestic animals, especially dogs, for its antineoplastic effects. In dogs there appears to be a narrow window of therapeutic safety, and other NSAIDs are considered safer alternatives for analgesia. Few studies of piroxicam's use in cats have been published.

OPIOIDS

Opioids produce their effects by binding with specific opiate receptors. Historically, veterinarians were reluctant to use this class of drugs in cats because opioids were reported to cause dysphoria, mania, and excitement. These effects were most likely dose-related or route of administration-related responses, and opioids are now routinely used for analgesia in domestic cats. Other adverse opioid effects in cats include respiratory depression, loss of thermoregulatory ability, sedation, nausea, vomiting, and mydriasis. Effects will vary with the agent and dosage, but large cats seem more susceptible to these nonanalgesic effects, on a comparable dose basis, than domestic cats. All opioids have the advantage of their effects being terminated by administration of an opioid antagonist, of which *naloxone* is the safest.

Morphine, *oxymorphone*, *hydromorphone*, and *meperidine* are mu-receptor agonist opiates that have been used in domestic cats for postoperative pain. Morphine is the agent most often associated with causing dysphoria or excitement in cats. All these drugs are controlled substances (U.S. Drug Enforcement Administration Category II), and their use in many practices has largely been replaced by the less stringently controlled opioid analgesics, buprenorphine and butorphanol. The latter agents, however, are not as potent as the pure mu-receptor analgesics.

Fentanyl is a mu-receptor agonist opiate that has gained widespread use as a postsurgical analgesic in domestic species because of its formulation in a transdermal delivery system (patch). Transdermal delivery offers the advantages of a long duration of delivery and effects (≥ 72 hours) and the ability to stop administration (by patch removal) if necessary. After patch application in domestic cats, there is a lag time of 6 to 8 hours before analgesic effects. As a result, additional analgesia is required to bridge the period between patch administration and onset of analgesia. Patches need to remain in good contact with the skin to be effective; this typically requires bandaging, which is difficult to maintain in nondomestic felids. As a result, fentanyl patches can be used only in select nondomestic felids.

Buprenorphine is a partial mu-receptor agonist with potent analgesic properties. It is available in the United States only in the injectable form, but this preparation may also be administered to cats transmucosally, via the oral mucous membranes. Onset of

analgesia after oral transmucosal administration is more rapid than when used intramuscularly and is a potential way to administer this drug following recovery from surgery.⁹ Buprenorphine has relatively few adverse effects when used at recommended dosages and has the distinct advantage of a long duration of activity (>6 hours).

Butorphanol is a mu-receptor antagonist that produces analgesia through its binding to kappa receptors. This combination of receptor affinities results in a *ceiling effect* to its analgesic and adverse effects, a dosage point above which there is no increase of effects. Butorphanol appears to provide analgesia to the viscera but poor body wall or muscular analgesia. In addition, butorphanol is relatively short acting (<2 hours), and effective analgesia after major surgery requires frequent redosing. Large cats seem particularly susceptible to butorphanol's sedative effects.

Tramadol, although structurally not an opioid, has weak affinity for mu receptors. Its principal mechanism of analgesic action appears to be by inhibiting reuptake of serotonin and norepinephrine within the spinal cord; as a result, tramadol appears to modulate pain at the spinal level. It does not affect COX enzymes and may be given safely in combination with NSAIDs. It has few adverse effects, although dose-dependent respiratory depression has been observed in anesthetized domestic cats. Tramadol appears to have an extremely low risk of dependency, but it is recommended that animals receiving long-term therapy be weaned off treatment slowly. Tramadol is not a controlled agent in the United States and is available only in an oral form.

TREATMENT OF ACUTE PAIN

Assessing the alleviation of postsurgical pain has been the primary model for studying treatment of acute pain in domestic cats. Although surgery is not a major part of nondomestic felid practice, elective and emergency procedures are performed, and all require postoperative pain management. Additionally, drug regimens developed for perioperative situations are applicable to episodes of acute pain.

Acute and postoperative pain is usually treated with injectable agents, frequently while the animal is anesthetized. Because of the frequent reluctance of cats to eat when recovering from surgery and the stress induced by repeated injections, those regimens that provide long postoperative duration of activity are preferred. Fortunately, studies of domestic cats sug-

gest that most analgesic drug regimens have relatively similar results after surgery. Opioids generally perform better during the first hours after surgery, whereas NSAIDs provide better analgesia after 4 hours post-surgery, likely because of NSAIDs' slower onset of action compared with opioids.

Most experts agree that preemptive analgesia (analgesics administered before start of surgery) provides superior benefits to the same agents administered after noxious stimuli. There is some debate whether NSAIDs truly have a preemptive effect on pain. However, whether their benefits are caused by preemptive effect or the onset of activity effect, most investigators support the administration of appropriate NSAIDs before surgery. The possibility of NSAIDs affecting renal blood flow mandates that if used preemptively, diligent efforts should be made to maintain blood pressure and renal perfusion during anesthesia.

Many studies have compared analgesics in cats during the immediate (within 20 hours) postoperative period. A comparison of postoperatively administered buprenorphine (0.006 mg/kg intramuscularly [IM]), meperidine (5.0 mg/kg IM), and ketoprofen (2 mg/kg subcutaneously [SC]) showed that ketoprofen provided the best analgesia during the 18 hours following ovariohysterectomy.¹⁰ Another study comparing buprenorphine (0.01 mg/kg IM), oxymorphone (0.05 mg/kg IM), and ketoprofen (2 mg/kg IM) administered after onychectomy, with and without subsequent sterilization, found that buprenorphine had the lowest cumulative pain scores for 12 hours after surgery.⁵ A comparison of postoperatively administered NSAIDs—carprofen (4 mg/kg SC), ketoprofen (2 mg/kg SC), and meloxicam (0.2 mg/kg SC)—after ovariohysterectomy showed very little difference between the treatment groups for 18 hours after surgery.¹¹

When compared with butorphanol, NSAIDs have provided superior analgesia. Preoperatively administered meloxicam (0.3 mg/kg SC) and butorphanol (0.4 mg/kg SC) were compared, following onychectomy, and cats treated with meloxicam had less lameness and less pain.³ When preoperative carprofen (4 mg/kg SC) was compared to postoperative butorphanol (0.4 mg/kg SC), both provided similar analgesia for cats undergoing ovariohysterectomy, but the carprofen group required fewer administrations of "rescue" analgesics than the butorphanol group.¹

Buprenorphine, 0.015 mg/kg IM after surgery, then 0.005 mg/kg IM every 8 hours (q8h) for three doses, has been used in a tiger undergoing abdominal surgery.⁶ Butorphanol (0.1 mg/kg SC) has been used for analgesia after repair of previous onychectomies in

tigers.⁴ I have routinely used butorphanol (4.0-5.0 mg [total dose] SC once) in subadult and adult lions and tigers after castration, with satisfactory short-term effects.

A few drugs have been investigated for use in the days after surgery.⁷ Ketoprofen (<5 days) and meloxicam (~14 days) are the only two NSAIDs typically recommended for short-term postoperative use in domestic cats (see Table 35-1).

Local and regional analgesia are important methods for perioperative pain relief in cats. Epidural morphine (Morphine Sulfate, 15 mg/mL, Abbott Laboratories, Chicago) can be used in large cats (>100 kg) undergoing laparotomies or caudal limb procedures. Typically, 10 mL of the solution is delivered in a manner identical to that used in domestic carnivores before surgery. The only adverse effect observed was dysphoria, in one tiger that received greater than 11 mL of morphine epidurally and additional parenteral morphine for pain.

TREATMENT OF CHRONIC PAIN

In contrast to the treatment of postsurgical pain, the treatment of chronic pain in cats has received minimal investigation. *Osteoarthritis* affects many geriatric nondomestic cats and is one of the most common reasons for long-term analgesic therapy in exotic felids. Long-term treatment of pain in these patients can improve their ability to move and their overall quality of life.

Aspirin, 10 mg/kg orally (PO) q72h, has been used successfully to treat osteoarthritis pain in a tiger and lion for months and over a year, respectively. No adverse effects were found in those animals at necropsy.

Meloxicam, 0.1 to 0.2 mg/kg PO or SC, 0.1 mg/kg q24h for 5 days, then 0.1 mg/kg PO q48-72h, has been used in a variety of nondomestic felids.¹² Our experience indicates that adequate analgesia may be achieved with lower doses or longer dosing intervals than those just cited (see Table 35-1) and that nondomestic felids can be maintained on meloxicam for months. Limited experience suggests piroxicam may be similarly effective, while considerably less expensive than meloxicam. Multiple-month therapy for treatment of pain has not yet been attempted with piroxicam.

Tramadol has also proved to be a very effective oral analgesic in lions, tigers, and a clouded leopard. It can be used for long-term therapy, and sedation and ataxia in animals given higher doses were the only adverse

effects encountered. Because it is not an NSAID, tramadol can be used more confidently in geriatric cats with renal disease or gastrointestinal problems. It may be used alone or in conjunction with an NSAID, such as meloxicam, in refractory animals.

Etodolac is an indole acetic acid derivative with selective COX-2 inhibition. It has not been recommended for use in domestic cats but has been used (5 mg/kg PO q48h for five doses, then q72h) to treat osteoarthritis pain in tigers.² There is an anecdotal report of a tiger developing gastric ulcers after etodolac treatment.

Adjunctive therapies such as weight reduction, nutraceuticals, rearrangement of exhibit "furniture," and other options should also be considered when treating feline osteoarthritis.

OTHER AGENTS AND GENERAL PRINCIPLES

A variety of other agents have been investigated for their analgesic effects, including some immobilization agents typically used for nondomestic felids. Ketamine and medetomidine, at subanesthetic dosages, have both been shown to have adjunctive analgesic effects. Additionally, certain tricyclic amine drugs, such as amitriptyline, traditionally used for treatment of behavior disorders in people and animals, are being investigated for treatment of chronic pain.⁸

CONCLUSION

Buprenorphine, carprofen, and meloxicam offer practitioners several good options for preemptive treatment of surgical pain in nondomestic felids. Because of its short duration of analgesic effects, butorphanol should be redosed at approximately 2-hour intervals, or as needed, when used for postsurgical analgesia. Ketoprofen and meloxicam are good choices for oral treatment of pain in the days following surgery. Meloxicam and tramadol appear to be the best agents at this time for the long-term treatment of chronic pain in nondomestic felids.

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CHAPTER 36

Mycobacterial Diseases in Carnivores

KAY A. BACKUES

Mycobacterial infections are uncommon but serious diseases in captive and free-ranging wild carnivores. Although susceptibility to *Mycobacterium* spp. is not considered high in this family of mammals, infections caused by the three major groups of pathogenic mycobacteria have been documented: *Mycobacterium tuberculosis* complex (*M. tb* complex), *Mycobacterium avium* complex (MAC), and saprophytic *Mycobacterium* spp. (atypical mycobacteria).^{1,2,5,6,21} The organ systems affected are related to route of exposure, virulence of the organism, and host susceptibility.^{1,10,12}

The severity of the disease and sequelae make the presence of mycobacterial disease in captive carnivores an important clinical entity. The many ramifications of mycobacterial infection in an individual captive carnivore encompass the institution's collection management, husbandry procedures, and staff safety measures, as well as monitoring and governmental regulatory compliance. Mycobacterial infections in free-ranging carnivores have been associated with introduction of the disease into the animal's habitat, primarily from human activities.^{1,12,25}

This chapter briefly reviews typical clinical disease findings in both captive and free-ranging carnivores, diagnostic procedures, and long-term medical monitoring after a diagnosis of mycobacterial infection has been made.

MYCOBACTERIUM TUBERCULOSIS COMPLEX

Clinical Findings and Transmission

The *M. tb* complex of bacteria contains the related species *Mycobacterium tuberculosis*, *M. bovis*, *M. microti*, and *M. africanum*. *Mycobacterium tuberculosis* complex has a wide host range, but historically, maintenance hosts of the disease have been primarily ruminants

and primates. Cases of *M. tb* complex have been documented in captive and free-ranging carnivores of the families Felidae, Canidae, Procyonidae, Mustelidae, and others.^{1,2,7,21,23} The majority of *Mycobacterium* isolates from the order Carnivora, including the family Felidae, have been *Mycobacterium bovis*.

The preponderance of *M. bovis* isolates in both captive and free-ranging carnivores is thought to result from ingestion of infected prey species and feeding on contaminated carrion or carcasses.^{1,12,2,25} In some cases, however, no clear source of infection could be determined.^{7,21}

Pulmonary, gastrointestinal, and hepatic infections of *M. bovis* have been seen in free-ranging African lions (*Panthera leo*) and cheetahs (*Acinonyx jubatus*).¹² Captive tigers (*Panthera tigris*), African lions, and snow leopards (*Uncia uncia*) have presented clinically with respiratory distress, unthriftiness, weight loss, and lameness.^{7,16,19,21} Pulmonary involvement has been the most consistently reported clinical finding in captive felids, with radiographic signs of bacterial pneumonia, cavitary lesions, multifocal nodules, pneumothorax, and partially collapsed lung; in one case, hypertrophic pulmonary osteoarthropathy of the bones of the forelimbs was noted.^{7,19,21,24} Hemogram changes are generally nonspecific signs of inflammatory disease; moderate leukocytosis, neutrophilia, monocytosis, and anemia. Serum chemistry profile abnormalities consisted primarily of hypoalbuminemia and hyperglobulinemia with occasional hypercalcemia.^{19,21} During clinical examination and at subsequent necropsy, mucopurulent exudate was seen in the trachea of several big cats.^{16,19,21,24}

Infected wild carnivores have been identified during epidemiologic surveys of live collected animals and from carcasses.^{2,12,25} In wild carnivores, identification of *M. bovis* infection has coincided with *M. bovis* infections in prey species.^{12,2,25}

When typing of *M. bovis* isolates in free-ranging carnivores and a feral domestic cat has been performed,

the isolates matched serovars found in the local maintenance host.^{11,25}

Carnivores are considered “spillover” rather than maintenance hosts for *M. bovis*, and long-term sustained infections in a carnivore population without continued reexposure to contaminated material from prey species has not been documented for most species.^{1,2,12} Transmission from one carnivore to another is also poorly documented both in captive and free-ranging animals, primarily because of the inability to differentiate between infections from repeated contact with an infectious source or true contagious spread between conspecifics.^{2,7,19,12}

Exceptions to the “nonmaintenance host” rule exist; one is the European badger (*Meles meles*) in England and Ireland.³ Infection in this species is maintained in the population through social contact and effective aerosol and cutaneous transmission, making the badger a true maintenance host.³ Once established, *M. bovis* infection appears to be progressive in carnivores.^{7,19,21}

Free-ranging lions are thought to have a comparatively fast progression of the disease and may quickly succumb to pulmonary or hepatic infection with *M. bovis*.¹² Captive large felids have also succumbed rapidly after onset of clinical signs.^{7,21}

Clinical cases of *M. bovis* reported in zoo collection carnivores have been limited, with few contact animals becoming infected. Once a case is documented in a facility, however, every attempt should be made to identify and eliminate other infected animals and find the source of the infection. The disease has been invariably fatal in carnivores; treatment of known infected animals is not recommended because of safety concerns for staff and potential for spread to contact animals.

Diagnosis

Antemortem diagnosis of *M. tb*-complex infection may be difficult because clinical presentation and organ systems affected are variable. The progressive nature of the disease and the animal's failure to show clinical signs until the disease becomes advanced often lead to cases being diagnosed at necropsy, by histologic examination, or through microbiologic methods.⁷ However, cultures may be negative despite gross and histologic evidence of the disease. In captive *Panthera* spp., antemortem diagnostics were successful in isolating the organism through bronchoscopic or transtracheal aspirates of tracheal exudates.^{16,19,21}

Indirect antemortem diagnosis may be worthwhile. However, several studies have shown the lack of value

of the intradermal tuberculin skin test for *M. tb* complex in carnivores.^{11,19,24} The use of polymerase chain reaction (PCR) has shown the presence of *M. bovis* genetic material in necropsy samples and may prove to be a useful adjunct to microbial culture attempts.⁷ A protein-A enzyme-linked immunosorbent assay (ELISA) has also shown some promise as a tool to screen *M. bovis* suspects and contact animals for exposure or potential disease.^{11,21} Protein-A ELISA used to screen both domestic cats and captive African lions as contact and noncontact animals suggested that some contact animals did develop antibodies to both *M. bovis* and *M. avium* purified protein derivative (PPD) antigens.^{11,21} Long-term follow-up with these animals revealed no evidence of clinical infection in the African lions and no histologic evidence at necropsy in the domestic cats.^{11,21} Results were at least suggestive that such non-species-specific ELISA tests could be useful in antemortem diagnosis of *M. bovis* infection in felids.

Other non-species-specific serum antibody tests, such as the multiple-antigen ELISA, multi-antigen print immunoassay (MAPIA), and lateral-flow technology (Rapid Test) have been developed for diagnosis of *M. tb*-complex infection in other nondomestic animals.^{17,20} MAPIA and Rapid Test have been adapted to assist in the detection of *M. tb*-complex disease in one species of carnivore, the European badger (*M. meles*), and may be attempted on sera from other carnivores and contact animals suspected of infection with *M. tb* complex. Unfortunately, these tests are not available commercially, although access may be possible through direct requests to research institutions working with these tests.

An antemortem test that is non-species specific and does not rely on host immune response is antigen 85 (Ag85). The Ag85 test has shown promise in detecting *Mycobacterium* infections in other wildlife species and may be useful in detecting infections in free-ranging and captive carnivores. Antigen 85 is a major secretory protein produced by actively growing mycobacteria. This test has become an adjunct test in human medicine to detect mycobacterial infections and recrudescence.¹⁴ Access to these diagnostic tests may be available through human testing services or by direct research request in some cases.

Surveillance and Epidemiology

A known infected animal should be euthanized and the premises disinfected with mycobactericidal disinfectants. Although aerosol transmission has not been documented between carnivore species, the frequent

presentation of pulmonary lesions makes this route of transmission possible. Therefore, contact animals should include those that shared the enclosure with the infected animal as well as those animals in immediately adjacent enclosures. Screening of contacts should be ongoing to prevent further spread of the disease in the collection. However, contact animals should not be assumed to be infected.^{11,21}

Contact and adjacent areas should be quarantined and no new animals added to these enclosures until the attending veterinarian and regulatory agencies are satisfied that *M. bovis* infection is no longer present in any of the contact carnivores. A contact animal that dies or is euthanized for other reasons should receive a full necropsy, with histologic examination of tissues and mycobacterial culture and isolation of any suspicious lesions.

Contact animals should receive long-term testing and surveillance. Repeated screening tests of contact animals should include complete physical examinations, complete blood counts (CBCs), serum chemistry panels, and radiographs of thorax and abdomen, as well as tracheal wash cytology with acid-fast staining and mycobacterial culture of the tracheal aspirate. Swellings, enlarged lymph nodes, and any draining tracts should be explored with fine-needle aspiration, impression smears or biopsy for cytology, and culture. The presence of acid-fast bacteria on tracheal wash cytology is not diagnostic for pulmonary *M. bovis* infection, but this finding warrants microbial culture, attempted PCR analysis if available, and repeat sampling. Serum and other specimens should be collected and banked for potential future diagnostic testing. Information from additional diagnostics (e.g., MAPIA, Rapid Test, multiple-antigen ELISA, Ag85), is needed to assist in refining antemortem testing for *M. tb* complex in captive and free-ranging animals. Screening tests may initially be done as frequently as every 6 months for the first year, then less often if no further cases are confirmed.

Isolation of *M. bovis* or other *M. tb*-complex bacteria from obtained samples is diagnostic for infection. Every effort should be made to differentiate the isolate by strain to facilitate determining the source and route of the infection's spread through the collection. *Mycobacterium* strain determination involves restriction fragment length polymorphism (RFLP) analysis of the mycobacterium's deoxyribonucleic acid (DNA).²⁵ Use of RFLP on isolates from a domestic cat and coyotes indicated that these infections were identical to those isolated from free-ranging and ranched cervids, respectively.^{11,25} Isolates of *M. tb* complex from free-ranging and captive carnivores should be submitted

for similar typing to better understand the epidemiology of *M. tb* complex and possibly identify the sources or reservoirs of infection.

Historical evidence heavily supports diet as the route of exposure. Therefore, in *M. tb*-complex infection of captive carnivores, investigations into food quality are warranted.^{2,12,19,25}

Zoonotic Potential

Mycobacterium bovis and other members of the *M. tb*-complex group are well-documented causes of tuberculous disease in humans.¹⁰ Infected carnivores should be considered a health risk to staff working with these animals. In one reported case of *M. bovis* in a captive African lion, three keepers developed positive skin test after exposure to the animal.²¹ It is at least theoretically possible that human caretakers could also be a source of infection for captive exotic carnivores. For the protection of both staff and animals, routine intradermal tuberculin skin testing of animal caretakers is a well-established health-screening tool in captive wildlife facilities and should be a consideration for personnel working closely with free-ranging animals as well.

MYCOBACTERIUM AVIUM COMPLEX AND ATYPICAL MYCOBACTERIA

The *M. avium* complex (MAC) and atypical *Mycobacterium* spp. are opportunistic saprophytic bacteria that are ubiquitous in the environment.^{8,15} Although MAC and atypical mycobacteria are not closely related, they may be grouped together as "nontuberculous mycobacteria." Some of the more important species in these groups are *M. avium*, *M. intracellulare*, and *M. scrofulaceum* in the MAC group and *M. fortuitum*, *M. kansasii*, *M. chelonae*, *M. abscessus*, *M. genavense*, *M. smegmatis*, and *M. haemophilum* in the atypical group. Atypical mycobacteria may be either fast or slow growing and, for species such as *M. genavense* and *M. haemophilum*, may be distinguished by their fastidious culture requirements.^{8,15} Many of these bacteria have been reported to cause infections in a variety of mammal, bird, reptile, and amphibian species, but disease in carnivores appears to be rare.^{10,15}

Clinical Findings and Transmission

Reports of MAC and atypical mycobacterial infection in carnivores are limited to domestic cats and a

pinniped.^{6,8} Clinical signs of infection have included cutaneous or subcutaneous masses with or without fistulous tracts, weight loss, emaciation, thickened intestines, vomiting, diarrhea, hematochezia, inappetence, and anemia.^{5,6,8,18} Hemogram findings in infected animals vary from leukocytosis to leukopenia with nonregenerative anemia and thrombocytopenia, and in one case of disseminated infection, acid-fast bacteria were seen in leukocytes on a blood smear.^{6,8,18} Gross necropsy lesions vary according to organ system affected, but caseous abscessation of mesenteric and regional lymph nodes, disseminated multifocal granulomas in parenchymous organs, and granulomas in the skin were common.^{5,8,18,26} Transmission of the organism is thought to occur by ingestion or through traumatic skin wounds.^{15,26} Infected animals are not considered to be directly contagious to other animals; the soil is the reservoir for these mycobacteria.^{8,15}

Diagnosis and Treatment

Diagnosis is often made when the disease is advanced and prognosis is poor in animals with disseminated infection. Biopsy, cytology of impression smears, and culture may yield an antemortem diagnosis when superficial lesions are seen.²⁶ Compared with tuberculous mycobacteria, the number of bacteria found in these lesions is often large and may be more easily found on microscopic examination of acid-fast stains of aspirates, impression smears, and biopsy.¹⁵ The non-species-specific and non-immune response-dependent Ag85 test, when correlated with other diagnostic procedures, may also hold promise for antemortem testing of MAC bacterial infections.¹⁴ Not all cases of nontuberculous mycobacterial infection are fatal.²⁶ Immunocompetent domestic cats with limited nodular cutaneous infections have recovered spontaneously or have been cured by surgical excision of lesions.²⁶ Various antibiotic regimens have also been attempted.²⁶

Surveillance and Epidemiology

Once infection with a nontuberculous *Mycobacterium* sp. is identified in a captive carnivore, animals in direct contact should be considered exposed to the same source but not necessarily infected. Decontamination of the environment should be attempted where applicable; enclosures should have dirt substrate removed; concrete and other hard surfaces should be disinfected with mycobactericidal disinfectants; and items such as branches and food bowls should be dis-

infected or discarded. Access to the enclosure should be limited and use of a footbath containing a mycobactericidal solution required.

Recommended screening tests for contact animals should include complete physical examinations, CBCs, and serum chemistry panels to look for nonspecific inflammatory changes. Survey radiographs and ultrasound may reveal enlarged internal lymph nodes. Nodules, draining tracts, and enlarged lymph nodes should be sampled by fine-needle aspiration, impression smear or biopsy for cytology, culture, and PCR testing. Serologic survey of captive felids for retroviral status is a routine part of preventive health programs in zoologic institutions but may be repeated if nontuberculous mycobacterial disease is identified in a felid. The organ system presentation of the index case may indicate other diagnostic tests for contact animals. When possible, excess biopsy material and serum should be stored for further testing, such as PCR and Ag85, respectively. Surveillance in the exposed population should continue, possibly for several years, because the rate of progression for these infections is unknown in most species of carnivores.²⁶

The close association between disease caused by these organisms and host immunosuppression must be considered. The majority of reports in domestic cats are associated with immunosuppressive retroviral infections. One case was reported in a feline renal transplant recipient receiving immunosuppressive chemotherapy.^{5,8,18} Immunosuppression resulting from stress was suspected in a fatal *M. smegmatis* infection in a California sea lion.⁶ Free-ranging and captive felids have been reported as being infected with immunosuppressive retroviruses.¹³ Numerous studies document wildlife exposure to environmental contaminants, with immunosuppressive effects that could manifest as mycobacterial infections.^{9,22} Infections caused by nontuberculous *Mycobacterium* spp. in both captive and free-ranging carnivores should prompt the veterinarian to look for potential causes of immunosuppression.

Zoonotic Potential

Humans seem similar to other mammalian species in that infection with nontuberculous mycobacteria is infrequent in the immunocompetent individual. However, nontuberculous *Mycobacterium* spp. are a major cause of disease and mortality in humans with immunosuppression caused by human immunodeficiency virus (HIV) infection or organ transplantation.^{8,10} Contamination of the environment with organisms shed from infected animals constitutes a potential

threat to exposed staff, and precautions should be taken when handling materials from these animals. If an outbreak is identified, the risk to exposed staff and possibly the public should be considered during cleanup procedures.

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CHAPTER 37

Meliodosis in Marine Mammals

REIMI E. KINOSHITA

Meliodosis is the disease caused by *Burkholderia pseudomallei* (previously *Pseudomonas pseudomallei*), a gram-negative bacillus. The closest relative to *B. pseudomallei* is *B. mallei*, the etiologic agent of glanders in horses, and a zoonosis. Unlike *B. mallei*, however, *B. pseudomallei* is ubiquitous in soil and water in endemic areas (of Southeast Asia and Northern Australia). *Burkholderia pseudomallei* was first identified in Burma in 1911 in a 10-year-old male morphine addict with lung abscesses.⁶⁰ It has subsequently become an important cause of community-acquired disease in humans in some endemic regions.^{10,14}

Meliodosis affects a wide range of animal species, including laboratory animals, livestock (pigs, sheep, goats, cattle), companion animals (horses, dogs, cats), zoo and wildlife animals (marsupials, camelids, primates, deer, birds), and even perhaps cold-blooded animals (crocodiles, snakes, tortoises).^{6,12,35,37,55} However, species susceptibilities to and manifestations of infection differ. Marine mammals appear to be especially susceptible; five species of cetaceans (*Tursiops gilli*, *Tursiops aduncus*, *Orcinus orca*, *Pseudorca crassidens*, and *Lagenorhynchus obliquidens*) and eight species of pinnipeds (*Zalophus californianus*, *Eumetopias jubatus*, *Arctocephalus pusillus*, *Neophoca cinerea*, *Phoca vitulina*, *Mirounga leonine*, *Hydrurga leptonyx*, and *Halichoerus grypus*) have been reported to have meliodosis.^{13,33} Almost all reported cases of meliodosis in marine mammals are from Ocean Park, an oceanarium in Hong Kong.

ETIOLOGIC AGENTS

Burkholderia pseudomallei is an aerobic, non-spore-forming, gram-negative bacillus that appears as short rods with bipolar staining, giving it a “safety pin” appearance. It has two or more polar flagellae and is motile. The organism grows on standard laboratory media such as blood agar and, more selectively, on

MacConkey agar.²² However, the selective medium of choice is Ashdown medium, which contains crystal violet and gentamicin.^{4,63} The morphology of colonies of *B. pseudomallei* may be smooth and mucoid or, more distinctively, dry and wrinkled after incubation for a few days. Colonies have a purple color if grown on Ashdown medium. The organism also has a pungent “earthy” odor and forms a pedicle when grown in broth. Biochemical tests used to identify *B. pseudomallei* include a positive oxidase reaction, production of gas from nitrate, activity of arginine dihydrolase plus gelatinase, and the oxidation of a variety of carbohydrates.²² The Rapid NFT commercial kit by BioMerieux, API 20NE, has been evaluated to be greater than 99% accurate in identifying *B. pseudomallei*.¹⁸ *Burkholderia pseudomallei* is a containment level 3 organism.¹

EPIDEMIOLOGY AND
EPIZOOTIOLOGY

Southeast Asia and Northern (tropical) Australia are the major endemic foci for meliodosis. Endemic areas generally lie between the latitudes of 20° N and 20° S and have a tropical or subtropical climate.²⁶ *Burkholderia pseudomallei* is an environmental saprophyte, surviving in soil and water, and often may be isolated from the environment in endemic areas, although the distribution of the organism is uneven. Climate (rainfall, temperature, sunlight); soil composition, type, and chemistry; and the interaction of *B. pseudomallei* with other biologic organisms (e.g., plants, other soil microflora) may affect the multiplication and survival of *B. pseudomallei* in its environment, thereby determining its distribution and the prevalence of disease. Moisture and temperature are two significant environmental factors. Endemic areas have high rainfall and temperatures, and both environmental *B. pseudomallei* and meliodosis are rare in countries with low rainfall.¹⁷

Infection is primarily through exposure to soil or water contaminated with *B. pseudomallei*.^{17,36} In humans the methods of natural infection are inoculation, inhalation, and possibly ingestion, with inoculation considered the most common. However, the mode of infection could be documented in only 5.7% of cases in Thailand.⁴⁹ The primary route of infection in marine mammals remains uncertain. The incubation period for melioidosis is also not known in marine mammals. Reports in humans range from 2 to 3 days²⁰ to “a few days to weeks.”¹⁵ It is believed that the acute fulminating septicemic form of melioidosis has a short incubation period and short course.⁵⁹

Melioidosis was first described in Hong Kong in an outbreak in dolphins at Ocean Park in 1975, resulting in the sudden death of 24 dolphins.²⁷ Human melioidosis started being recognized in Hong Kong after this first report,^{45,61} although the prevalence of disease is apparently low, with an estimated 10 persons/year diagnosed in the territory.²⁵ Available records show that at least 78 marine mammals have died from melioidosis at Ocean Park since it started maintaining these animals in 1974. This number is probably underestimated because it does not even include the 24 dolphins cited in Huang’s paper.²⁷ Only cases that could be definitively diagnosed (i.e., positive culture of *B. pseudomallei*) were included in epidemiologic studies, and records for these dolphins were limited. These mortalities reflect the scale, importance, and significance of melioidosis at this facility. Epidemiologic investigations continue to be conducted at Ocean Park³³ to try to understand the disease better and formulate preventive measures. Findings include the following:

1. Almost annual occurrence of melioidosis in marine mammals since 1976, suggesting a continued presence and exposure to *B. pseudomallei*.
2. More than one third of all mortalities in cetaceans attributed to this disease.
3. No statistically significant increased risk with gender and location in cetaceans at Ocean Park (records for pinnipeds not complete enough to analyze).
4. Strong seasonal distribution, with 95% of all marine mammal melioidosis cases occurring during Hong Kong’s summer monsoon season (May to October).

Increased prevalence of disease during the wet monsoon season in endemic regions is also a typical finding in human cases.^{14,29,49} Cases in animals have also been associated with flooding in Australia.^{30,31} To determine the prevalence of *B. pseudomallei* in the envi-

ronment, intensive sampling of soil and fresh water within Ocean Park, especially around marine mammal facilities, was done. This resulted in isolation rates of about 1.4% in soil, 14% in fresh water, and 0% in seawater. For the rest of Hong Kong, isolation rates have been about 1.5% in soil and 14% in fresh water.³³ These environmental isolation rates are similar both within and outside Ocean Park. This suggests that the organism is distributed relatively evenly throughout Hong Kong and is not concentrated at this facility. The prevalence of *B. pseudomallei* in soil and water in Hong Kong is considered low when compared to northeastern Thailand, which has both a high soil isolation rate of 50% to 68% and a high prevalence of human melioidosis.^{44,56,62}

Subjective observations at Ocean Park revealed that melioidosis cases in marine mammals occurred shortly after severe tropical cyclones or typhoons, and the stronger the typhoon, the greater the risk of developing disease. This observation was supported by the isolation of *B. pseudomallei* from moisture collected from strong winds during typhoons. Findings from environmental isolation studies at Ocean Park, although still circumstantial, suggest that strong winds and rain might carry and move *B. pseudomallei* from distant sites to expose susceptible animals. The organism has only been isolated (from wind and rainwater) within marine mammal facilities at Ocean Park during inclement weather, and it has not been isolated from soil or water near these facilities. The human literature also reports similar associations; a cyclone in Western Australia was followed by the first cases of acute melioidosis in a previously nonendemic area.²⁹

CLINICAL FINDINGS

Humans and Other Animals

Clinical manifestations of melioidosis may be variable, thereby earning its nickname of the “great imitator.” Descriptions in humans include an acute “disseminated” septicemia with a short course, often resulting in death despite treatment; a pulmonary form; and a chronic form presenting with abscessation or granuloma formation in almost any organ system.¹⁶ However, it is believed that the majority of infections in people must be mild or asymptomatic because up to 49% of some populations in endemic areas have developed antibodies to *B. pseudomallei*.³² Patients who develop septicemic melioidosis are acutely critically ill, have reduced consciousness, are pyrexia and diarrheic, and have multiorgan involvement, typically of

the lungs, liver, and spleen.⁵ The pulmonary form may develop either as a primary pneumonitis secondary to septicemia or more chronically as a cavitating pneumonia. Localized infections of other organs and organ systems include skin, subcutis, muscles, joints, glandular tissue, lymphatic tissue, bone, genitalia, urinary tract, and central nervous system.

In other animals, acute septicemia, pulmonary lesions, and abscessation in virtually any organ system have also been reported. Abscesses and microabscesses, which may be described as "nodular," are mainly distributed in the liver, spleen, lymph nodes, and lungs of affected animals. The lesions are often enclosed within a fibrous capsule.³⁵ Lameness resulting from suppurative polyarthritis is relatively common,^{12,50,51} and nodular purulent lymphangitis causing lameness has also been described.⁷ Other presentations include mastitis, especially in goats and sheep^{12,38,39}; orchitis and periorchitis^{7,21}; and aortic aneurysms.¹² Transplacental infection causing abortions has been described.^{30,52} Asymptomatic infection of livestock in Australia appears to be a common outcome, with melioidosis diagnosed by isolating *B. pseudomallei* from abscesses during routine meat inspection.^{31,51}

Marine Mammals

Almost all cetaceans present with acute septicemic melioidosis. The clinical presentation of these cases was nonspecific and included an acute onset of anorexia, pyrexia, and lethargy. Pyrexia appears to be a consistent early indicator of disease. Dyspnea and extreme dullness with inactivity were often observed terminally. Occasionally the cetacean was found dead in the pool or presented at a terminal stage. After the onset of clinical signs, 29 animals died in about 5 days (mean). Hematology usually demonstrated an initial neutrophilia with a left shift, with or without a concurrent leukocytosis, and often leukopenia before death. Liver enzymes (alanine transaminase, aspartate transaminase, lactate dehydrogenase, gamma-glutamyltransferase) were often elevated and alkaline phosphatase was decreased on serum chemistries. The closest to the chronic localized form of melioidosis was in a bottlenose dolphin with osteomyelitis of the lumbar vertebrae and extensive abscessation around and erosion of the dorsal spinous processes of the tenth and eleventh thoracic vertebrae. This male dolphin had a 5-month history of a decreased appetite, weight loss, and later reduced mobility of the spine. He eventually succumbed to disseminated septicemic melioidosis.

In pinnipeds, as in cetaceans, most cases at Ocean Park presented as an acute septicemia. The clinical presentation was nonspecific and included reluctance or refusal to perform, lethargy and dullness, tachypnea, diarrhea, and partial to complete anorexia. Pyrexia was present in all eight cases for which body temperature was obtained and recorded. The mean time to death after the first clinical signs for most pinnipeds was about 5 days (range, 1-23 days). Hematologic and serum biochemical findings were similar to those in cetaceans. One atypical case was a female California sea lion (*Zalophus californianus*) that displayed signs of localized melioidosis. She presented 41 days before death with inappetence, dullness, and abscessation (thick, yellow/cream-colored purulent material cultured positive for *B. pseudomallei*) and cellulitis of the mammary glands and surrounding subcutis. Despite treatment, which included surgical drainage, debridement, placement of indwelling drains, flushing of the site, and fluid plus antimicrobial therapy, the sea lion developed septicemic melioidosis and died. Hematology and serum chemistry revealed a neutrophilia with a left shift and an increasing leukocytosis throughout the sea lion's illness, with progressive anemia and hyperglobinemia. The duration of malaise and the development of extensive pockets of abscessation in the mammary tissue and subcutis suggest chronic localized melioidosis.

PATHOLOGY

The typical pathology of melioidosis in humans and animals consists of focal nodular lesions, histopathologically defined as microabscesses or granulomas and ranging in size from microscopic to several centimeters in diameter (Figures 37-1 and 37-2). These lesions may be distributed in any organ, although in acute disseminated melioidosis, lesions are distributed widely, with the lung, liver, spleen, and lymph nodes mainly involved.⁴⁰ In chronic cases, lesions are usually localized and confined to a single organ. Hemorrhage and congestion are often associated with these lesions, which may vary from a soft to a firm consistency with a purulent center and may coalesce to form large, irregular abscesses or granulomas.

In marine mammals at Ocean Park the liver, spleen, and lung were the main organs with grossly visible lesions. Pathology in these organs included white-cream to yellow necrotic foci randomly distributed throughout the parenchyma, miliary to several centimeters in diameter; organomegaly; edema; and petechial to ecchymotic hemorrhages. Other common

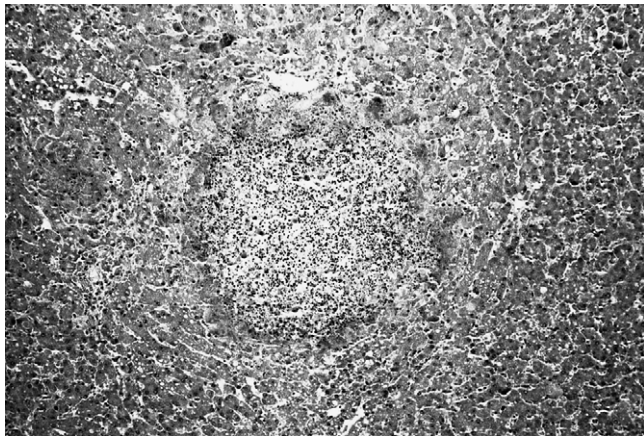


Fig 37-1 Low-power view of necrotizing hepatic granuloma from false killer whale, comprising central necrosis surrounded by rim of macrophages (hematoxylin and eosin stain, $\times 125$).

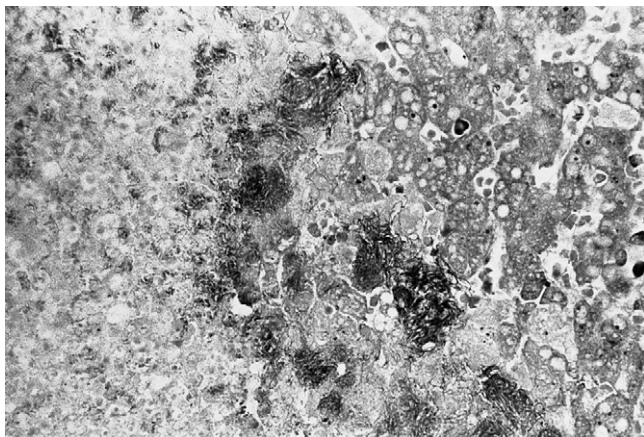


Fig 37-2 High-power view of edge of liver granuloma showing filamentous bacteria associated with macrophages (Giemsa stain, $\times 500$).

but nonspecific findings included reactive and edematous lymph nodes, gastric ulceration, and hyperemia of the intestinal mucosa. The typical histologic lesion, regardless of tissue type, was focal necrosis, microabscesses, hemorrhage, fibrin exudation, and a variable accumulation of polymorphonuclear neutrophils, again mainly distributed in the liver, spleen, and lung, in that order.²⁴ In about one third of marine mammal cases examined histologically, slender gram-negative bacteria were associated with these necrotic foci. These pathologic findings are consistent with those of acute septicemic melioidosis in other species. It has been suggested that in humans and other species of animals the nodular lesions increase in size with illness, and the duration or chronicity of disease could be deduced from the size, degree of encapsulation, and consistency of these nodules.^{23,40,53} Both the clinical and the pathologic findings in marine mammals at Ocean Park

suggest that cetaceans have a shorter disease course than pinnipeds.

Burkholderia pseudomallei was isolated from the majority of organs sampled at necropsy in marine mammals at Ocean Park: heart blood (66/67), liver (65/68), lung (63/66), spleen (62/66), lymph nodes (35/39), kidney (47/54), brain (7/9), pancreas (3/3), and adrenal glands (3/3), almost always as a pure culture. The organism was also isolated as part of a mixed culture in 32 of 42 (10/24) stomach and 29 of 41 (3/3) intestinal content samples.

DIAGNOSIS

A definitive diagnosis of melioidosis is made by positive culture of *B. pseudomallei*. The bacterium is not easily identified or isolated from nonsterile sites without the use of selective agar plates or selective enrichment broths. It may also be easily dismissed as a pseudomonad contaminant. As previously noted, the selective medium of choice is Ashdown medium, which contains crystal violet and gentamicin.^{4,63} Selective broths are effective and useful in limiting overgrowth by other bacteria. Suspicious colonies may be reliably identified using the API 20NE biochemical tests.¹⁸

Unlike in humans, from whom *B. pseudomallei* is often isolated from sputum samples, purulent discharges, or from blood in septicemic cases,⁶⁴ the organism has been relatively rarely isolated antemortem from Ocean Park's marine mammal cases. Before 2000, despite repeated blowhole and fecal samples, as well as infrequent blood cultures, from cetaceans that died from melioidosis, *B. pseudomallei* had only been isolated from the blowhole of one dolphin and the blood of another dolphin before death. Since 2000, however, all 13 marine mammals diagnosed definitively with melioidosis were positive on antemortem blood culture. *Burkholderia pseudomallei* was also isolated from gastric (three dolphins) and fecal samples (two pinnipeds and one dolphin) from these 13 cases. Improved isolation protocols through the use of enrichment and selective broths and multiple and frequent blood cultures accounted for the organism being isolated more often from clinical samples after 2000. Blood for culture is now obtained as soon as deviation from normal behavior or pyrexia is observed during the melioidosis season. Isolation and definitive identification of *B. pseudomallei* using the API 20NE biochemical tests takes about 6 days. Early diagnosis and treatment would improve the outcome of infection.

Two tests have been adopted at Ocean Park that may give a presumptive diagnosis of melioidosis, even

within 24 hours of a blood culture being obtained. A latex agglutination test using a monoclonal antibody to detect *B. pseudomallei* antigen is being used either on blood culture fluid or on suspicious colonies detected on media.³ The test has specificity and sensitivity values approaching 100%, it is simple to perform, and results are immediate. The other test is a direct immunofluorescent antibody test, which is also highly specific and sensitive.⁵⁷

An enzyme-linked immunosorbent assay (ELISA) has recently been developed to detect antibodies to *B. pseudomallei* in marine mammals.¹¹ This test is based on a sensitive and specific ELISA for humans using the *B. pseudomallei* exopolysaccharide antigen.^{2,19} Preliminary results show promising sensitivity and specificity in marine mammals.¹¹ Serodiagnosis would be valuable in diagnosing nonbacteremic cases in which the foci of infection were unknown. The ELISA has been found to be of limited use for early diagnosis because it has taken up to 18 days for seroconversion in a definitively diagnosed case of melioidosis. However, this ELISA is valuable in identifying animals with presumptive melioidosis, which would undergo long-term treatment (eradication therapy) necessary to prevent relapse. It may also be used for serologic monitoring and possible detection of subclinical cases.

TREATMENT AND MANAGEMENT

The first successful treatment of a culture-positive case of melioidosis in a marine mammal at Ocean Park was in 2001. Previously, all marine mammals with melioidosis died from the disease. Even in humans with septicemic melioidosis managed with the antibiotic therapy of choice and intensive care, mortality is still 40% to 50%.⁸ The antibiotic of choice for septicemic melioidosis in humans is ceftazidime, although imipenem and the newer carbapenems such as meropenem show promise.^{8,43}

Cetaceans

Currently for cetaceans, ceftazidime is administered at 30 mg/kg three times daily (tid) intravenously (IV), combined with ciprofloxacin at 20 to 25 mg/kg twice daily (bid) orally (PO) or enrofloxacin 5 mg/kg once daily (sid) intramuscularly (IM), for a minimum of 2 weeks but based on normalization of, or acceptable, blood parameters for a week. Eradication therapy involves amoxicillin-clavulanic acid at 10 mg/kg every 8 hours (q8h) and amoxicillin at 6.5 mg/kg q8h for a

minimum of 20 weeks. This antimicrobial regimen is similar to that described in humans with septicemic melioidosis. The preferred eradication therapy in humans with the conventional four-drug therapy of trimethoprim-sulfonamide, chloramphenicol, and doxycycline is replaced by amoxicillin-clavulanic acid, which is often used in children and pregnant women because of fewer side effects.⁴⁸ Trimethoprim-sulfonamide in combination with ceftazidime may further reduce mortality in humans.⁴⁶ However, severe bone marrow suppression developed in three dolphins treated with trimethoprim-sulfonamide in combination with ceftazidime.³⁴ Quinolones (used in combination with ceftazidime, as per our current protocol) have not proved to be effective in the treatment of melioidosis, although these agents may have prophylactic uses. Some human clinicians believe that the clinical response with this combination is better than with sole therapy using ceftazidime or the carbapenems. Supportive care (e.g., fluid/nutritional therapy) and liver protectants are also provided.

The current protocol for treatment of melioidosis in cetaceans must be interpreted with caution because it is based on few subjects. Only two blood culture-positive and three serologically diagnosed cases of melioidosis have been treated using this drug regimen. Of these, one of the culture-positive cases died despite treatment. The protocol will surely evolve based on new cases, information, research, and experience. One dolphin with blood culture-positive melioidosis was treated with meropenem IV but died after 24 days from the onset of clinical signs. A severe phlebitis at the antibiotic administration site was seen at necropsy.

Pinnipeds

In septicemic pinniped cases, meropenem at 18.5 mg/kg tid subcutaneously (SC) and trimethoprim-sulfadiazine at 25 mg/kg bid PO/IM, supplemented with folic acid (25-50 mg/pinniped bid PO), for 2 to 4 weeks (based on clinical response and blood parameters) is the current initial antibiotic regimen. Eradication therapy follows the conventional four-drug therapy in humans: trimethoprim-sulfadiazine at 25 mg/kg bid, chloramphenicol at 12.5 mg/kg bid, and doxycycline at 7.5 mg/kg bid, supplemented with folic acid (25-50 mg/pinniped bid PO), for a minimum of 20 weeks. Six positive-blood culture cases (four harbor seals and two California sea lions) and one serologically diagnosed case (harbor seal) of melioidosis have been treated with meropenem and minor variations of the described protocol. All survived except for one California

sea lion, which developed bone marrow suppression and finally died from the resulting complications. No pinniped has been successfully treated for melioidosis with ceftazidime given IM.

RISK FACTORS AND PREVENTIVE STRATEGIES

Disease is the outcome of a dynamic relationship between the host and the agent that is affected by three main factors. Each of these components and their interaction may be assumed to affect the prevalence of melioidosis in marine mammals (Figure 37-3). One of these factors is the properties of the infectious agent itself. Molecular characterization of *B. pseudomallei* at Ocean Park and in Hong Kong suggests that strains affecting marine mammals at Ocean Park are not especially virulent or infectious.³³ The interaction of exposure (quantity and method) and host immunity/susceptibility to *B. pseudomallei* probably determines the outcome of infection in marine mammals. In humans, risk factors have been established, including diabetes mellitus (most significant), renal disease, excessive alcohol intake, cirrhosis, steroid therapy, thalassemia, chronic lung disease, occupational exposure, age, and consumption of kava.^{9,10,41,47,49} Diabetes mellitus, renal disease, and excessive alcohol intake all may lead to impaired polymorphonuclear cell func-

tions, which might be the critical defense against developing melioidosis.¹⁴

It has been suggested that the common denominator of these risk factors may be metabolic derangement²⁸ and warrants further research. I believe that host immunity (species and individual) is the strongest determinant of the three factors in determining susceptibility to and outcome of infection by *B. pseudomallei* in marine mammals. The organism is opportunistic in humans and endemic in the environment, and low-level exposure of all marine mammals to the organism is likely, yet cases are usually sporadic. Environmental conditions could also contribute by affecting the degree of exposure of the host to *B. pseudomallei*.

Prophylactic strategies at Ocean Park have been directed at both improving host immunity and reducing exposure. A vaccine against melioidosis produced specifically for Ocean Park's marine mammals was used starting in 1987⁵⁴ but did not appear to offer protection and thus was discontinued in 1997. No vaccine against *B. pseudomallei* has yet been developed for humans, although trials are in progress.⁵⁸ Changes in husbandry practices have been implemented, mainly since 1993, including strict hygiene, optimum nutrition, improvements in water quality and monitoring, and considerations for management of the social grouping, demands, and interactions of marine mammals for their physical and mental well-being. A preventive

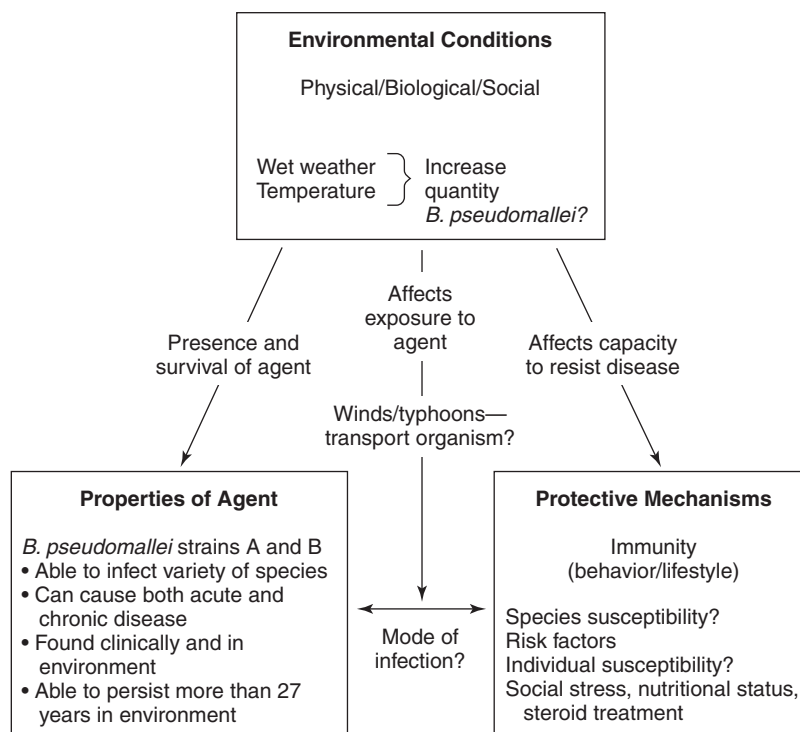


Fig 37-3 Interaction of factors influencing development of melioidosis in marine mammals at Ocean Park oceanarium, Hong Kong.

health program was developed. Close monitoring of health indices includes daily body temperatures, weekly body weights, and monthly blood sampling, obtained through operant conditioning. Health parameters are vigilantly assessed to allow management changes and early intervention or treatment. Finally, based on results from environmental isolation studies, "typhoon shelters" have been erected in all marine mammal facilities to reduce exposure to *B. pseudomallei* during inclement weather.

The prevalence of melioidosis in cetaceans at Ocean Park has decreased since 1993 from an average of 2.4 cases/year (1974–1992) to 0.6 cases/year (the records/inventory of pinnipeds at Ocean Park were not complete enough for prevalence to be determined). However, other factors, such as selection of *Tursiops aduncus* that appear to have better survivorship⁴² and minimal importation of new cetaceans, also likely contributed to this decrease in incidence.

CONCLUSION

Melioidosis is a severe disease in marine mammals that typically presents with acute septicemia and is associated with high mortality. Marine mammals appear to be particularly susceptible to *Burkholderia pseudomallei*. Although the disease has a limited geographic distribution and has only been reported as a problem in a single marine institution, it has created a unique opportunity to study an interesting disease in marine mammals. This may help in further understanding the dynamics, possible mechanisms, and immune response to disease in general in marine mammals. Melioidosis also may have public health implications because infected animals may contaminate their environment, or animal products may contain *B. pseudomallei*.

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Inflammation in Marine Mammals

THOMAS H. REIDARSON

The most common illnesses affecting marine mammals result from infectious agents that cause inflammation. Similarly, a number of less common, noninfectious diseases are equally capable of eliciting similar levels of inflammation. Armed with these clues, the clinician must be able to identify a morbid individual within a group of animals who are adept at masking clinical signs of illness. Frequently, blood samples are taken from suspicious individuals and judgments are made based on seemingly insignificant changes in hematologic and biochemical values.

This chapter defines the hallmark changes in clinical pathology indicative of inflammation and provides several new insights in identifying individual marine animals that might otherwise remain undetected.

DIAGNOSTIC TESTS

Currently, the most reliable tests that indicate the presence of inflammation are white blood cell (WBC) counts, including differential counts and reticulocyte counts; hemoglobin or packed cell volume; fibrinogen; erythrocyte sedimentation rate; albumin; alkaline phosphatase; and iron.^{8,10} Almost all these tests are affected variably by all known inflammatory diseases of marine mammals.

Most of us as clinical veterinarians are familiar with total WBC and differential counts because they change with infection and the resulting inflammation. In an effort to detect early onset of inflammation more closely, we have redefined the criteria for “band” neutrophils. Rather than the more conventional definition (i.e., cells with pinched filaments >50% of filament diameter), we count any neutrophil with chromatin spanning the lobules as a band.

The reason for this new definition is simple. Although we use other indicators to make a diagnosis

of inflammation, our goal is not to miss individuals who are in early stages of band production and not experiencing an obvious left shift. In the past we would have overlooked this early cell, so by redefining bands, we now observe 2% to 5% in normal individuals. By using the more conventional definition for bands, we would more likely miss early subclinical individuals. As with other inflammatory parameters, the astute clinician should weigh the importance of this finding with other changes in clinical pathology and in concert with the history and physical examination. The fundamental goal is to avoid the surprise of finding a significant change in the percentages of bands when a second blood sample is taken shortly after the first.

For the vast majority of marine mammal cases involving acute inflammation, *hemoglobin* (Hb) and *packed cell volume* (PCV) decrease an average of 7% to 10%. The reason is not entirely understood, but fluid shifts in response to the release of inflammatory mediators are presumably involved, rather than an actual blood loss. Once the inflammatory nidus is controlled, these parameters return to normal, generally without evidence of a regenerative hemogram. If, on the other hand, Hb and PCV slowly drop to subnormal levels, in the absence of responsiveness (i.e., PCV, mean cell volume, and reticulocyte counts increase, and polychromatic cells appear), *anemia of chronic disease* (ACD) is most likely, again rather than blood loss.

In animals with chronic inflammation and low-grade anemia, *reticulocyte counts* generally drop, most likely because of decreased red blood cell (RBC) regeneration. This must be distinguished from blood loss anemia, in which reticulocyte counts rise above normal while Hb and PCV continue to drop. As with other subjective tests (e.g., differential count), values for normal reticulocyte numbers must come from marine mammals under the veterinarian’s care.⁸

In my experience, *plasma fibrinogen* is the most reliable indicator of inflammation as long as the test is quantitative and not qualitative (as with heat precipitation methods). Some clinicians believe fibrinogen correlates directly with *erythrocyte sedimentation rate* (ESR); in my experience, however, variable effects are seen. In fact, fibrinogen levels are almost always elevated when the ESR is increased, but the converse is frequently not true. This is especially evident with dehydrated patients, in which increased viscosity slows the sedimentation of RBCs, but has little or no effect on the concentration of fibrinogen.

Serum *albumin* levels usually drop below normal from the direct effect of inflammatory mediators on the synthesis of messenger ribonucleic acid (mRNA) in the liver.^{13,14} The drop normally follows a rise in fibrinogen, which is rarely as dramatic; however, a 10% to 20% decline is expected with presence of inflammation. Globulin levels normally remain unchanged in the early stages of infections and generally increase if the process becomes chronic.

Serum *alkaline phosphatase* (ALP) levels vary with age, nutritional status, and presence of infection or inflammation. In cetaceans, ALP is extremely high early in life because of intense bone remodeling, and only malnutrition, inflammation, and infectious agents may reduce these levels. Even older individuals with significantly lower baseline levels, weight loss or gain, or extrahepatic infection may produce a corresponding increase and decrease in levels of ALP. As with serum albumin, synthesis of mRNA in the liver appears to be sensitive to these effects. Unlike in most other mammals, ALP in marine mammals is not affected to the same extent as alanine transaminase (AST), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), and lactate dehydrogenase (LDH) in inflammatory hepatic diseases.

During an acute phase of a bacterial infection, serum *iron* may drop rapidly and rather dramatically. Iron is sequestered by iron-binding proteins and temporarily stored in the liver, rendering it unavailable for invading bacterial pathogens. Acute-phase proteins (e.g., interleukin-1, C-reactive protein, prostaglandins, tumor necrosis factor, interferon) help mediate this iron sequestration.² The opposite is true of hepatocellular bacterial infections and even some noninfectious diseases, such as hemochromatosis, lipidosis, and azole hepatopathies, in which iron is liberated from the liver and into the serum.¹⁰ Trends in serum iron levels are important when evaluating clinical condition and prognosis, although changes in magnitude do not appear to correlate directly with severity.

SAMPLE COLLECTION

To run all the available tests for inflammation, blood must be drawn into collection tubes containing ethylenediaminetetraacetic acid (EDTA) or heparin for complete blood count (CBC) and differential, in citrate tubes for extrinsic clotting profile and fibrinogen, and in thrombin for biochemistries. Because most cetaceans are factor XII (Hageman factor) deficient, the intrinsic pathway is not functional; therefore, activated coagulation time (ACT) and activated partial thromboplastin time (APTT) tests are inaccurate. For the same reason, blood should always be drawn into thrombin-containing collection tubes to provide the necessary matrix for clotting. In contrast, clotting occurs slowly and organizes poorly in non-thrombin-containing tubes.

Almost all the important hematologic and biochemical tests may be analyzed from a volume of blood as little as 1.25 mL placed in citrate-containing tubes.⁹ The first test must be a CBC, followed by centrifugation to harvest plasma for fibrinogen and biochemistries. To compensate for the citrate diluent, all parameters must be multiplied by a factor of 1.036 or 1.016 for a 1.25-mL or 1.5-mL sample, respectively. Multipliers are not necessary for volumes greater than 1.5 mL. The only unreliable values using this method are calcium, sodium, and carbon dioxide, and because of the small volume, ESR tests cannot be run. Although unconventional, the ability to run an entire suite of inflammatory parameters with less than standard volumes of blood is extremely valuable, especially with patients who cannot or will not present for larger volumes.

EFFECTS OF STRESS

The effect of stress-related hormones resulting from handling, restraint, and stranding has been studied in various marine mammal species. A slower-onset type of stress-mediated leukocytosis with neutrophilia, eosinopenia, and lymphopenia caused by adrenocortical secretion has been described in a number of marine mammals, including net-captured ringed seals, restrained northern elephant seals, captured beluga whales, and dolphins after simulated transport.² Other adrenocortical stress-related changes in marine mammals include decreased hematocrit and ALP and increased cortisol, adrenocorticotrophic hormone (ACTH), and insulin.¹⁶ Although not well documented in marine mammals experiencing chronic illness, these changes presumably account for debilitation,

characterized by declining body condition, weight loss, and anemia.

DEHYDRATION AND REPERFUSION

Unless a patient's illness is detected early before the onset of inappetence, many present with some degree of dehydration. As the patient becomes hemoconcentrated, sludging significantly slows the ESR and to a lesser extent raises all the concentration-dependent analytes (fibrinogen, albumin, ALP, iron). If the clinician is relying on the typically reviewed parameters to assess inflammation, patients with mild inflammation may be overlooked.

Once the patient is rehydrated, a number of inflammatory parameters increase, including WBC count, ESR, and fibrinogen, with a corresponding decrease in Hb, iron, and albumin.⁵ The phenomenon of reperfusion injury after substantial injuries or invasive surgery is well recognized.¹² With sufficient damage to a body part, whether the result of trauma, infection, or pressure (e.g., stranded whale or dolphin lying on a beach for some time), reperfusion of affected tissues may push endotoxins and reactive oxygen species into the systemic circulation. This may trigger a cascade of systemic inflammatory changes.

NONINFECTIOUS CAUSES OF INFLAMMATION

Although uncommon in marine mammals, certain types of neoplasia may incite an inflammatory cascade.⁷ For example, urogenital carcinoma of California sea lions (*Zalophus californianus*) typically present with the hallmark hematologic and biochemical changes observed with infectious disease. Even though a gamma-herpesvirus is linked to pathophysiology of this cancer, its contribution to inflammation is minor compared to the necrosis associated with tumor growth and invasion of internal organs.¹⁷

Forms of trauma, especially involving muscle or gastrointestinal (GI) tract, lead to inflammatory reactions from tissue damage.^{1,3} Release of tissue thromboplastins, RBC fragments, peroxidases, and other muscle cell membrane fragments may incite an inflammatory cascade.¹⁹ Furthermore, if shock were to ensue, GI barriers may become compromised, leading to translocation of bacteria and toxins across membrane barriers.^{4,6,18} Any of these processes may also stimulate

production of local and systemic cytokine and other inflammatory mediators.

INFLAMMATION ASSOCIATED WITH PREGNANCY

Inflammatory changes are frequently seen in blood work taken from dams in the last 2 to 3 weeks of gestation.^{11,15} In our experience, these include all the parameters mentioned except reticulocyte count. If, on the other hand, these changes occur much earlier, the end result is generally fetal distress, premature birth, or abortion. In all cases, it is difficult to determine whether the inflammation is a result of an infectious agent or the cascade of events involved in pregnancy. Implantation, endometrial development, abortion, and parturition are mediated by hormones, interleukins, and cytokines. The main causes of inflammation appear to be interleukin-6 (IL-6) and C-reactive protein, which produces cervical ripening and softening.

ASSESSING INFLAMMATION

General guidelines for assessing the presence of illness in marine mammals include age, clinical history, onset of clinical signs, environment, social issues with conspecifics, level of hydration, accuracy of diagnostic equipment, and proficiency of medical technologists. Most illnesses seen in marine mammals involve inflammation, whether the diseases are infectious, traumatic, neoplastic, or shock related.

When assessing an animal with a suspected inflammatory disease, a number of conditions should be met. A diagnosis should not rest solely on clinical pathology; rather, historical, physical, and diagnostic findings should corroborate one another. On occasion, when routine physical examinations reveal abnormal inflammatory parameters, the clinician's knowledge of physiology and pathophysiology is tested. Before reaching the conclusion that a patient has an infectious disease, it is important to check the reliability of the laboratory tests, determine whether the abnormal values are appropriate for the individual and the species, and most importantly, "first do no harm."

In my experience, close monitoring of certain inflammatory products helps guide decisions on initiating and discontinuing therapy. Both fibrinogen and IL-6 levels mirror one another during an inflammatory response. These levels rise quickly early in the disease process and remain elevated as long as

inflammation is present. Once inflammation is controlled and fibrinogen and IL-6 levels return to baseline, a decision to discontinue therapy may be made. Interleukin-6 is a relatively new biologic test that unfortunately takes several days to complete. For this reason, fibrinogen is more useful, and IL-6 remains a valuable retrospective, corroborative test. If a clinician is considering a new test, it is imperative to determine whether it uses species-specific reagents and is validated with nondomestic species because nonvalidated tests may frequently produce erroneous results, leading to misguided decisions.

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Capture and Anesthesia of Otariids in the Wild

ALBERTO PARÁS

Otariids are widely distributed in coastal regions throughout the world. The family Otariidae, in the order Pinnipedia, includes sea lions and fur seals (14 species in seven genera). As a group, they are distinguished from the true seals by their use of the fore flippers as the principal means of propulsion. Sea lions, which have broader muzzles and lack underfur, are generally larger than most fur seals. All species of otariids are gregarious and sexually dimorphic; males are substantially larger and darker in color than females.

Some of the populations of these species are relatively small, such as the Galápagos fur seal (*Arctocephalus galapagoensis*), the Galápagos sea lion (*Zalophus wollebaeki*), the isolated California sea lion (*Zalophus californianus*) population found in the Sea of Cortez in Mexico (20,000-30,000 individuals), and the Guadalupe fur seal (*Arctocephalus townsendi*), with no more than 8000 individuals. These populations have experienced significant reduction in size attributed to bacterial and viral epizootic diseases, contamination, the introduction of exotic species, competition with humans, and decreased food resources resulting from climatic phenomena such as El Niño Southern Oscillation events, which have caused up to 99% mortality in pups.¹⁵

Otariids are high-metabolism predators that consume species that aggregate in dense schools (sardines, anchovies, mackerel, hake) and normally are associated with upwelling zones where abundant food maintains populations as great as millions of individuals.¹ In several monitoring programs of marine ecosystem health, the study of otariids as sentinel species has intensified in the past few decades, including research on population dynamics, ecology, and interactions of these animals with the fisheries. The direct handling of otariids has been carried out since the 1980s using physical restraint and more recently with anesthesia, to mark, weigh, and measure animals and collect biologic samples. The study of adult females reflects local environmental conditions because they remain for

most of the year near the reproductive areas. With increased longevity, the raising of offspring may extend up to 1 year or more, and thus a good body condition of the female reflects in general a good health condition of the pups. The health study of pups offers a practical alternative to indirect evaluation of the condition of the mothers and the general condition of the colony.¹¹

Field studies that require the close manipulation of otariid females and pups call for effective techniques that have only a minor impact on the individuals and the breeding rookeries. The capture and anesthesia of individuals in field conditions differ in some ways from methods with captive animals; the history of the animal is unknown, their weight and physiologic status must be estimated, a fast recovery is required, and often the work is done under adverse climatic conditions. Normally the goals of the studies are to obtain accurate morphometric measurements and biologic samples, to measure physiologic data, and to attach biotelemetry equipment (Figure 39-1).

This chapter addresses the capture and anesthesia of free-ranging otariids.

UNIQUE ANATOMY AND PHYSIOLOGY

Otariids present certain anatomic and physiologic particularities that are important to consider. The body is long and hydrodynamic with a well-developed neck; pectoral flippers are relatively long; and otariids have strong caudal flippers that may rotate forward. The thin and highly vascular flippers play an important role in thermoregulation, especially with the underfur and thick subcutaneous fat layer (3-4 cm [1.2-1.6 inches]) in the rest of the body.

Otariids have specialized adaptations related to the amazing diving they perform (Figure 39-2), such as highly efficient lungs for gas exchange, higher blood



Fig 39-1 Gas anesthesia in Galápagos sea lion (*left*) and California sea lion (*right*), to attach biotelemetry equipment.

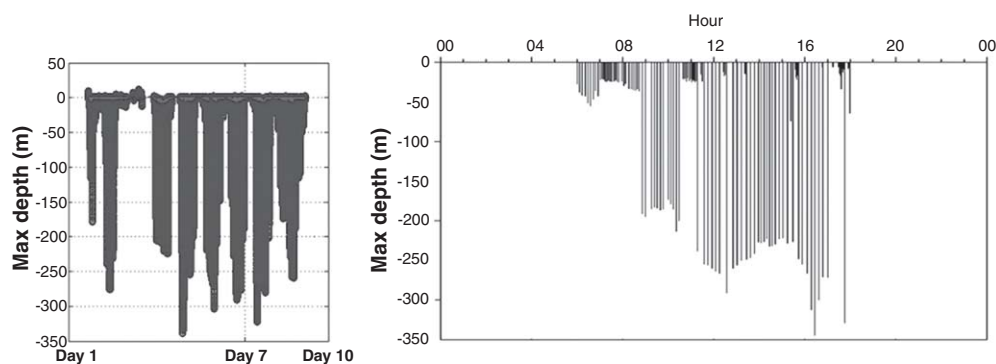


Fig 39-2 Dive record of female Galápagos sea lion using time-depth recorder during 1 day (*right*) and 10 days (*left*). (Courtesy Dan Costa.)

volume with an increased capacity to transport oxygen, and muscles rich in myoglobin. When submerged, otariids exhibit a dive response that involves the following²:

1. Apnea interrupts respiratory gas exchange and forces the use of blood and tissue oxygen stores to sustain aerobic metabolism (oxymyoglobin in the muscles).
2. Bradycardia decreases cardiac output and causes reflex peripheral vasoconstriction that maintains normal arterial blood pressure.
3. Body temperature is lower.
4. In long dives the energy metabolism switches to anaerobic, resulting in the rapid formation of lactic acid.

Cardiovascular adaptations in the venous system, such as the large hepatic sinus formed from the hepatic

veins, works as a large-capacity blood reservoir, which is presumed to assist in the shunting of circulating blood only to vital organs during a dive.⁴

Other anatomic particularities are the long tongue, which is poorly mobile; reduced mandibular mobility; prominent arytenoid cartilages; a small, flexible epiglottis; and the trachea, which bifurcates in the region of the thoracic inlet.¹²

Some of the common complications during restraint and anesthesia related to the unique anatomic and physiologic characteristics of otariids are head trauma, hyperthermia, apnea with hypoxemia and bradycardia, respiratory alkalosis, and endobronchial intubation.

CAPTURE AND HANDLING

Physical restraint of these animals in the field presents many challenges because of the long hydrodynamic

body, the size and strength of the adults, the need to control the head and the pectoral flippers, and the high incidence of hyperthermia during capture. The environment in which otariids live also presents challenges; for example, in some areas, such as desert climate and islands with difficult access to rocky beaches, the research protocol may require the capture of a specific individual from hundreds of animals over rough terrains or the transport of an individual by land or sea to the research camp.

The pups may be captured by lifting the animals by the base of the caudal flipper and maintaining the animal far from one's body. They may be placed on a table in sternal recumbency, and three people may manage the animal, handling the head, the pectoral flippers by placing them over the back of the animal to prevent them from having contact with the table, and the caudal flippers by pulling them backward.

In the case of juveniles or adult females, a hoop net must be used. The net is made from small mesh and needs to be long enough to permit the animal to be fully enclosed, with a hole at the bottom of the net that allows the nose and mouth of the animal to stick out, at the same time serving as a muzzle to prevent the animal from biting. This hole is also useful for the induction of gas anesthesia. The bottom of the net should have two layers of mesh to blind the animal and keep it calm. The right diameter and cone shape of the net will prevent the pectoral flippers from being used, limiting the movement of the animal. It is helpful to be able to disconnect the top of the net from the handle and to close it to prevent the animal from backing out of the net. To carry an animal inside a net by land or boat, a stretcher must be used; animals should never be dragged, because of the potential for head trauma. A stretcher is also useful if weighing is desired.

Males of otariid species are rarely handled physically because of their size and strength. To work with them safely, a trap cage must be built on land or at sea on a floating platform, where they may be secured before induction of anesthesia.

In cases in which pups are the main subject, it is best to collect several animals at the same time and keep them in a corral with a shade, cooling them frequently with water. This corral may also serve as a recovery area before returning them. In my experience, working with 24 pups for 12 minutes each will take up to 6 hours.

Before the start of any procedure, it is important to review the plan and the necessary equipment. The team must include personnel with knowledge on the species biology to minimize the disturbance to the

colony. The physical characteristics of the location (time, temperature, shade, wind, substrate, tide) must also be considered.

ANESTHESIA MONITORING

Respiratory depression, apnea, hypoxemia, and bradycardia are the most common complications encountered with injectable and gas anesthesia in otariid species. From the onset of the anesthesia, it is necessary to evaluate the palpebral reflex, capillary refill time, mandible tone, cardiac and respiratory rates, body temperature, and relative oxyhemoglobin saturation.

The respiratory rates tend to increase and stabilize during the anesthesia procedure, but significant hypoxemia (oxyhemoglobin saturation <75%) is often present during the induction. Oxyhemoglobin saturation will increase as the plane of anesthesia deepens and the apneas disappear.

The resting body temperature varies from 36.5° to 37.5° C (97.7°-99.4° F), the respiratory rate varies from 6 to 14 cycles/min, and the average heart rate in adult females is 55 beats/min and in pups is 90 to 180 beats/min.²⁰ The physiologic parameters to be expected during isoflurane anesthesia are body temperature from 36.5° to 41.5° C, respiratory rate from 8 to 32 cycles/min, oxyhemoglobin saturation from 75% to 97%, and heart rate in adult females from 60 to 98 and in pups from 75 to 210 beats/min. The heart rates tend to be higher during hyperthermia.¹⁴

Because of the dense hair coat and thick subcutaneous fat layer of otariid species, hyperthermia is a common complication. The time of day and duration of the capture also contribute to the problem. Some species, such as the Galápagos sea lion, apparently have a lower body temperature than other species so hyperthermia at a lower temperature must also be considered for this species. Keeping the flippers of the animal wet is a routine cooling procedure in the field.

INTRAVENOUS ADMINISTRATION AND VENIPUNCTURE

Access to the vein is always necessary to administer drugs and substances for research purposes or to obtain blood samples. Several veins are fairly accessible and may be used in different situations:

The caudal gluteal vein is found deep in the gluteal musculature, a few centimeters lateral to the sacral vertebrae and one third the distance from the palpable femoral trochanter to the base of the tail.¹⁹ This vein



Fig 39-3 Jugular venipuncture in adult female California sea lion.

is useful for repeated administration and sampling. To access the caudal gluteal vein, the animal is placed in sternal recumbency and the needle inserted perpendicularly, applying negative pressure to the syringe while slowly introducing the needle.

The jugular vein is well developed and deep in the neck musculature. It is not as visually apparent in otariids as in other terrestrial mammals, but it may be accessed for repeated administration and sampling and may be catheterized more easily than the caudal gluteal vein, although this is not an easy task. For the jugular venipuncture, the animal is placed in lateral recumbency, and the needle (18-20 gauge, 22-50 mm) is inserted obliquely into the musculature in pups or perpendicularly in larger animals; a middle or cranial site on the neck is preferred (Figure 39-3).

Other intravenous sites are the interdigital vein, which may be approached by the dorsal surface, between the radiating phalanges.¹⁹ The veins of the tongue or the tongue itself may be useful for the administration of drugs to counteract undesirable anesthetic effects.

CHEMICAL IMMOBILIZATION

Injectable drugs for the induction of anesthesia in otariid species have a narrow margin of safety, and these agents are rarely used in free-ranging otariids. Only in situations in which the animal is secured before anesthesia induction and during recovery (to prevent the animal from going into the water) might researchers choose to use injectable drugs with a remote delivery system. If the animal is captured with

a net, induction with gas anesthesia using a portable anesthesia machine is advised.

In the event of choosing an injectable anesthetic, the combination of tiletamine-zolazepam has worked well as an induction drug, at a dose of 1.8 to 2.5 mg/kg. Continuing the procedure with gas anesthesia,^{8,10} the recovery time may be lengthy. The more recent anesthesia protocols for captive otariids are medetomidine-midazolam-butorphanol,¹⁸ medetomidine-ketamine, medetomidine-zolazepam-tiletamine,^{5,6} and ketamine-diazepam.¹⁷

INHALATION ANESTHESIA

The use of halothane, isoflurane, or sevoflurane as an anesthetic agent for handling pups, juveniles, and adults in the field has been successful in several species of otariids, providing a quick induction, an ideal state of immobilization, a high margin of safety, and a complete and quick recovery.^{3,7-9,14,16,22}

For the application of the anesthesia, a portable anesthesia machine designed for field work is required, with a 3- to 7-L rebreathing bag for adult females and 1-L bag for pups. The system must be able to administer up to 10 L/min of oxygen for adult females and must have a leveling system. A tripod works well for the different terrain encountered (Figure 39-4). It is important to remember to level the vaporizer before introducing the gas anesthetic and to check the leveling frequently throughout the procedure. The vaporizer must be drained before moving the machine.

Pups are restrained manually, and isoflurane is administered at 5% in oxygen (1-2 L/min) for induction.

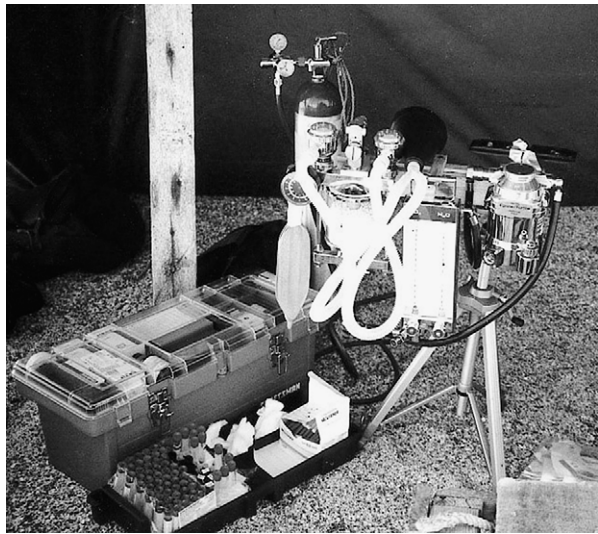


Fig 39-4 Portable anesthesia machine with tripod as leveling system.

A plastic anesthesia cone for foals is the correct size for the pups because it covers the eyes, calming the animal (Figure 39-5). An endotracheal tube is placed as soon as the animal is relaxed, by direct visualization with a laryngoscope, and the isoflurane concentration is adjusted according to stimuli response and the presence or absence of palpebral reflex and endotracheal tube tolerance. The animals stay in sternal recumbency, except when moved to facilitate the taking of measurements and samples. The time of induction will vary from 30 seconds to 3 minutes, depending on the weight and excitement of the animal. The anesthetic planes may be maintained with 1.5% to 2% isoflurane. The nutritional status and health of different populations of a species in a region vary widely. This will be reflected in differences in the average weights, difficulty of handling, time of induction, and concentration

of isoflurane needed to maintain the desired state of anesthesia.

In the case of juveniles and adult females, the induction is done with the animal inside the net, placing a plastic mask (modified highway road cone or anesthesia cone for foals), connected to the anesthesia machine, and delivering isoflurane at 4% to 5% in oxygen. The induction time will vary depending on the excitement and apneas normally present in the animal as soon as the mask is placed. For example, the Galápagos sea lion, a calm species, would be induced with isoflurane in 5 minutes, in contrast to the California sea lion and Galápagos fur seal, which are more stressful species and would take 20 to 25 minutes for induction. In these latter species the application of intravenous propofol as an induction agent at 1 mg/kg, applied 5 minutes after the start of the induction, has been useful to accelerate the induction, allowing endotracheal intubation 1 to 2 minutes after a successful venipuncture. The induction phase is the most critical part of the procedure, and everything needs to be ready to avoid hyperthermia and stress. Some animals may be apneic for short periods, slowing the induction and causing a marked bradycardia and a decrease in blood oxyhemoglobin saturation. When the animal holds back its breathing longer than a minute during the induction, it is stimulated physically and would resume breathing and return to the normal physiologic values. This behavior has been observed in animals with a more active temperament, such as fur seals.

Once relaxed, the animal is taken out of the net, and an endotracheal tube is placed to assist in the case of apnea or to prevent complications in the case of vomiting or regurgitation, which may be a secondary effect to the anesthetic or the animal's position. This is a common complication in lactating pups.



Fig 39-5 Induction of anesthesia in Galápagos fur seal pup using plastic anesthesia cone for foals.

For the endotracheal intubation, a large laryngoscope blade (35 cm) is used. The arytenoid cartilage is used to identify the larynx opening, and only 10 to 15 cm of the endotracheal tube is introduced in an adult animal to prevent endobronchial intubation.¹² Endotracheal tubes with an internal diameter of 4 to 6 mm are used for pups, 14 to 18 mm for adult females, and 22 to 26 mm for males.^{3,7,13,22} Sea lions and fur seals normally are very sensitive to intubation, so they need to be in a deep plane of anesthesia to tolerate it. Also, the characteristics of the saliva may partially obstruct the endotracheal tube, and during the procedure the tube may need to be cleaned or changed. The use of intramuscular atropine (0.02 mg/kg) has been recommended as a preanesthetic agent in captive animals, although in a field situation its use is not always practical.

Although otariids will maintain the breathing reflex during anesthesia, there are several reports of spontaneous apneas during anesthesia.^{3,7} Endotracheal intubation, positive-pressure ventilation, and a respiratory stimulant have been used to prevent apneic episodes. Doxapram has been used to stimulate respiration during general anesthesia at a dose of 100 mg intravenously, with variable results. Although it does stimulate ventilation and will speed awakening from narcosis, doxapram also will stimulate the coughing reflex and secretion of mucus.²¹ The administration of doxapram in the tongue or intratracheally by endotracheal tube may be an option for quick administration, but its efficacy has not been well established.

Some of the adverse effects of isoflurane in sea lions and fur seals are apneas, irregular breathing, respiratory and cardiovascular depression, and interference with thermoregulation.^{7,22} The advantages of gas anesthesia are ventilation of the animal in case of apneas, administration of oxygen, control of the depth of anesthesia with easy elimination of the anesthetic gases, a fast recovery without side effects, and the security that the animal may return to the water without risk. In cases in which injectable drugs are used for the induction, gas anesthesia is always recommended for maintenance.

ANESTHESIA RECOVERY

For the recovery from anesthesia, the animal is kept on oxygen for a few minutes and extubated before it starts resisting the endotracheal tube, then is left undisturbed. The animal will start having strong, deep breaths, a sign of recovery, and if left alone would actually fall asleep. This provides enough time for the

anesthesia to wear off; some animals will need to be awakened. By this time they will go into the water showing no signs of dissociation. In some animals that wake up sooner, showing some signs of dissociation, they are permitted to go into water rather than putting them through the stress of physical restraint and hyperthermia. The time of recovery in pups is less than 2 minutes and in females is 5 to 10 minutes.

CONCLUSION

The long-term health assessment of populations of free-ranging sea lions and fur seals attempts to document changes in the prevalence of infectious agents and toxins to interpret the population dynamics of these species. This requires well-planned capture and anesthesia procedures that will provide for safe handling of animals with little disturbance to the colony, a quick induction, an ideal state of immobilization that may require up to 2 hours of anesthesia, a high margin of safety, and a complete and quick recovery.

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Tissue Cyst-Forming Coccidia of Marine Mammals

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PARASITE PREVALENCE

Toxoplasma gondii

The main tissue cyst-forming coccidia reported from marine mammals are *Toxoplasma gondii*, *Sarcocystis neurona*, and unidentified *Sarcocystis* spp. parasites. The earliest report of *T. gondii* infection in marine mammals dates back 55 years to a captive sea lion residing in Pennsylvania.⁷² Since that time, *T. gondii* has been reported from wild and captive marine mammals from North America, Hawaii, South America, Europe, Asia, and Australia (Table 40-1). Based on the global distribution of *T. gondii* in cats, it is reasonable to suspect that marine mammals in other areas are also exposed.

Even arctic- and subarctic-dwelling seals and walrus (*Odobenus rosmarus*) with low exposure to cats have serologic evidence of *T. gondii* infection, prompting concerns regarding consumption of uncooked marine mammal tissue by indigenous human populations.^{30,32,50} Other subarctic species, such as Alaskan sea otters (*Enhydra lutris kenyoni*), have a lower prevalence of infection by *T. gondii* than their southern counterparts (*Enhydra lutris nereis*).^{36,61}

A wide range of marine mammals has been shown to be infected, including otariids, phocids, cetaceans, mustelids, and sirenians (see Table 40-1). However, the significance of the tissue cyst-forming protozoa to some marine mammal populations was not fully appreciated until the past decade. Based on current diagnostic testing, at least 36% of southern sea otters presenting for necropsy are infected with *T. gondii*, and 60% or more are seropositive.^{14,30,61,62} Protozoal infections caused by *T. gondii* and *S. neurona* are also a major cause of otter death, responsible for 8.5% to 23% of southern sea otter mortality.^{42,78}

Reports of *T. gondii* infection in marine mammals range from incidental to severe and fatal, and striking differences are noted when the various studies are organized by marine mammal taxa. Clinically signifi-

cant *T. gondii* infections are reported most frequently from southern sea otters and odontocete cetaceans. In contrast, *T. gondii*-associated disease is uncommon in otariids and phocids and is often associated with concurrent disease or immunosuppression.^{34,37,81} This low prevalence of *T. gondii*-related pathology is unlikely to result from low surveillance because rehabilitation and necropsy programs for otariids and phocids have existed worldwide for many years. However, mild or asymptomatic infections are easily missed.

Interestingly, reports of *T. gondii*-associated infection and mortality are nonexistent for mysticetes (baleen whales). This finding may reflect difficulty obtaining optimal samples for protozoal detection from large whales during necropsy, or low susceptibility of mysticetes to *T. gondii*. Some mysticetes forage near the shoreline and ingest large volumes of water, filter-feeding prey and sediment; thus exposure to *T. gondii* and *S. neurona* seems likely, and further investigation is needed.

Sarcocystis neurona

Sarcocystis neurona, the major causative agent of equine protozoal myeloencephalitis (EPM), was first reported as a pathogen of marine mammals over the past decade.* In contrast to *T. gondii*, reports of confirmed *S. neurona*-associated marine mammal mortality have thus far been limited to the Pacific Coast of North America (see Table 40-1), in areas where Virginia opossums (*Didelphis virginiana*) have been introduced. The reasons for this localized occurrence of infection are unclear because opossums capable of shedding *S. neurona* sporocysts are present throughout much of North and South America. Marine mammals with concurrent *T. gondii* and *S. neurona* infections are often reported

*References 12, 27, 29, 42, 45, 48, 49, 59, 60, 75.

Table 40-1

***Toxoplasma gondii*, *Sarcocystis neurona*, *Sarcocystis* spp., and Unidentified Protozoan Parasite Exposure in Marine Mammals**

Protozoan and Marine Mammal Species	Wild or Captive	Tests for Detection	Parasite-Associated Disease?	Animal Location	Reference
<i>Toxoplasma gondii</i>					
Phocids					
Harbor seal (<i>Phoca vitulina</i>)	Wild	HP	Yes*	Alaska	Van Pelt and Dietrich, 1973
		HP	Yes*	California	Gulland et al, 1997
		S	No	Washington	Lambourn et al, 2001
		S, ISO, PCR	No*	California	Miller et al, 2001a
		S	No	Alaska	Dubey et al, 2003b
		S	No	Eastern Canada	Measures et al, 2004
		ISO, PCR, SUB	No*	California	Conrad et al, 2005
Ringed seal (<i>Phoca hispida</i>)	Wild	S (all seroneg)	No	Northeast Atlantic	Oksanen et al, 1998
		S	No	Alaska	Dubey et al, 2003b
Harp seal (<i>Phoca groenlandica</i>)	Wild	S (all seroneg)	No	Northeast Atlantic	Oksanen et al, 1998
		S (all seroneg)	No	Eastern Canada	Measures et al, 2004
Bearded seal (<i>Erignathus barbatus</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Spotted seal (<i>Phoca largha</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Ribbon seal (<i>Phoca fasciata</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Hooded seal (<i>Cystophora cristata</i>)	Wild	S	No	Eastern Canada	Measures et al, 2004
Gray seal (<i>Halichoerus gryphus</i>)	Wild	S	No	Eastern Canada	Measures et al, 2004
Monk seal (<i>Monachus schauinslandi</i>)	Captive	HP, S, ISO, PCR, O	No	Eastern Canada†	Gajadhar et al, 2004
Elephant seal (<i>Mirounga angustirostris</i>)	Wild	HP, IHC, S, PCR, SUB	Yes	Hawaii	Honnold et al, 2005
	Wild	HP, IHC	Unknown	California	Dubey et al, 2004
Otariids					
Sea lion (<i>Zalophus californianus</i>)	Captive	HP	Yes	Pennsylvania	Ratcliffe and Worth, 1951
	Captive	HP	Yes	Hawaii	Migaki et al, 1977
	Wild	S	No	Alaska	Dubey et al, 2003b
	Captive	HP, IHC	Yes	South Carolina	Dubey et al, 2003b
	Wild	ISO, PCR, SUB	No*	California	Conrad et al, 2005
Fur seal (<i>Callorhinus ursinus</i>)	Wild	HP, IHC	Yes*	California	Holshuh et al, 1985
Walrus (<i>Odobenus rosmarus</i>)	Wild	S	No	Alaska	Dubey et al, 2003b

*Significant concurrent disease noted, including morbillivirus infection, trauma, bacterial sepsis, infection with other protozoal parasites, or other pathology.

†Experimental infection of seals, cats, or opossums.

HP, Histopathology; IHC, immunohistochemistry; S, serology; EM, electron microscopy; TEM, transmission electron microscopy; ISO, parasite isolation; PCR, polymerase chain reaction, sequencing, or other characterization techniques; SUB, genotyping; O, other, such as mouse bioassay; experimental infection of cats, opossums, or other marine mammals; or evaluation of patterns using epidemiologic techniques.

Table 40-1—cont'd

Toxoplasma gondii, Sarcocystis neurona, Sarcocystis spp., and Unidentified Protozoan Parasite Exposure in Marine Mammals

Protozoan and Marine Mammal Species	Wild or Captive	Tests for Detection	Parasite-Associated Disease?	Animal Location	Reference
Toxoplasma gondii —cont'd					
Odontocete cetaceans					
Killer whale (<i>Ocinus orca</i>)	Captive, wild	S	No	Japan	Murata et al, 2004
	Wild	S, PCR (all neg)	No	Japan	Omata et al, 2006
Pilot whale (<i>Globicephala melaena</i>)	Wild	S (all seroneg)	No	Spain	Cabezon et al, 2004
False killer whale (<i>Pseudorca crassidens</i>)	Captive	S (all seroneg)	No	Japan	Murata et al, 2004
Beluga (<i>Delphinapterus leucas</i>)	Wild	HP	Yes*	Quebec	DeGuise et al, 1995
	Wild	HP, IHC, S	Yes	Quebec	Mikaelian et al, 2000
	Captive	S	No	California	Dubey et al, 2003b
Bottlenose dolphin					
(<i>Tursiops truncatus</i>)	Wild	HP,IHC	Yes*	Florida	Inskeep et al, 1990
	Wild	HP, IHC, TEM	Yes	Florida	Cruickshank et al, 1990
	Wild	HP	Yes	Italy	DiGuardo et al, 1995a, 1995b
	Wild	HP	Yes*	Florida	Schulman et al, 1997
	Wild, captive	S	No	California, Florida	Dubey et al, 2003b
	Wild	HP	Unknown*	Florida	Dubey et al, 2003b
	Wild	S	No	Spain	Cabezon et al, 2004
	Captive	S	No	Japan	Murata et al, 2004
	Wild	S	No	Florida, South Carolina	Dubey et al, 2005
	Captive	S (all seroneg)	No	Japan	Murata et al, 2004
(<i>T. truncatus aduncus</i>)	Wild	HP, IHC	Yes	Australia	Jardine and Dubey, 2002
		S	No	Solomon Islands	Omata et al, 2005
<i>T. truncatus</i> hybrid (2)	Captive	S	No	Japan	Murata et al, 2004
Risso's dolphin (<i>Grampus griseus</i>)	Wild	HP, S	Yes	Italy	DiGuardo et al, 1995a
		HP, IHC	Yes	Spain	Resendes et al, 2002a
		S (all seroneg)	No	Spain	Cabezon et al, 2004
Humpbacked dolphin (<i>Sousa chinensis</i>)	Wild	HP, IHC, TEM	Yes*	Australia	Bowater et al, 2003
Common dolphin (<i>Delphinus delphis</i>)	Wild	S	No	Spain	Cabezon et al, 2004
Striped dolphin (<i>Stenella coeruleoalba</i>)	Wild	HP	Yes*	Spain	Domingo et al, 1992
		HP, S	Yes*	Italy	DiGuardo et al, 1995a, 1995b
		S	No	Spain	Cabezon et al, 2004
White-sided dolphin (<i>Lagenorhynchus obliquidens</i>)	Captive	S (all seroneg)	No	Japan	Murata et al, 2004
Spinner dolphin (<i>Stenella longirostris</i>)	Wild	HP, IHC	Yes*	Hawaii	Migaki et al, 1990
Porpoise (<i>Phocoena phocoena</i>)	Wild	S	No	Spain	Cabezon et al, 2004
Tucuxi (<i>Sotalia fluviatilis</i>)	Wild	HP	Yes	Brazil	Bandoli and De Oliveira, 1977

Continued

Table 40-1—cont'd

Protozoan and Marine Mammal Species	Wild or Captive	Tests for Detection	Parasite-Associated Disease?	Animal Location	Reference
<i>Toxoplasma gondii</i>—cont'd					
Mysticete cetaceans					
Minke whale (<i>Balaenoptera acutorostrata</i>)	Wild	S (all seroneg)	No	Northeast Atlantic	Oksanen et al, 1998
Sirenians					
Manatee (<i>Trichechus manatus</i>)	Wild	HP	Yes*	Florida	Buergelt, 1983
		HP	Possibly	Guyana	Dubey et al, 2003b
Mustelids					
Sea otter (<i>Enhydra lutris</i>)	Wild	HP	Yes	California	Thomas and Cole, 1996
		HP, S, ISO, PCR, SUB, O	Some cases [†]	California	Cole et al, 2000
		HP, IHC, PCR, O	Yes [†]	Washington	Lindsay et al, 2001
		HP, IHC, S, ISO	No	California, Washington, Alaska	Miller et al, 2002a
		HP, IHC, O	Some cases	California	Miller et al, 2002b
		S, O	No	California, Washington, Alaska	Hanni et al, 2003
		S	No	California, Washington	Dubey et al, 2003b
		HP, IHC, O	Some cases	California	Kreuder et al, 2003
		HP, IHC, S, PCR, SUB, O	Some cases	California	Miller et al, 2004
		HP, IHC, S, ISO, PCR, O	Some cases	California	Kreuder et al, 2005
		HP, S, ISO, PCR, SUB, O	Some cases	California	Conrad et al, 2005
River otter (<i>Lutra canadensis</i>)	Wild	S	No	NC, USA	Tocidlowski et al, 1997
		S, ISO, PCR, SUB	No	California	Miller and Conrad ⁵⁷
Ursids					
Polar bear (<i>Ursus maritimus</i>)	Wild	S (all seroneg)	No	Canada	Philippa et al, 2004
<i>Sarcocystis neuropa</i>					
Odontocete cetaceans					
Porpoise (<i>Phocoena phocoena</i>)	Wild	S, ISO	Unknown	California	Zabka and Conrad ⁸⁴
Phocids					
Harbor seal (<i>Phoca vitulina</i>)	Wild, captive	HP, IHC, S, TEM	Yes	California	Lapointe et al, 1998
	Wild	HP, IHC, S, ISO, PCR	Yes	California	Miller et al, 2001a
	Wild	HP, IHC	Yes	California	Colegrove et al, 2005

Table 40-1—cont'd

Protozoan and Marine Mammal Species	Wild or Captive	Tests for Detection	Parasite-Associated Disease?	Animal Location	Reference
<i>Sarcocystis neurona</i>—cont'd					
Mustelids					
Sea otter (<i>Enhydra lutris</i>)	Wild	HP, IHC	Yes	California	Thomas and Cole, 1996
	Captive	HP, IHC	Yes	Oregon	Rosonke et al, 1999
	Wild	HP, IHC, S, PCR, O	Yes	California	Lindsay et al, 2000
		HP, IHC, PCR	Yes	California	Lindsay et al, 2001
		HP, IHC, S, ISO, PCR	Yes	California	Miller et al, 2001b
		H, IHC, S, ISO, PCR, O	Yes [†]	California, Washington	Dubey et al, 2001b
		S	No	California, Washington	Dubey et al, 2001a
		HP, IHC, S, O	Some cases	California	Kreuder et al, 2003
		HP, IHC, S, ISO, PCR, O	Some cases	California	Kreuder et al, 2005
<i>Sarcocystis</i> spp. and <i>S. canis</i>-like spp.					
Phocids					
Harbor seal (<i>Phoca vitulina</i>)	Wild	HP	No	Alaska	Hadwen, 1922
Ringed seal (<i>Phoca hispida</i>)	Wild	HP	No	Alaska	Migaki and Albert, 1980
Monk seal (<i>Monachus schauinslandi</i>)	Captive	HP, IHC, TEM	Yes	Hawaii	Yantis et al, 2003
Bearded seal (<i>Erignathus barbatus</i>)	Wild	HP	No	Alaska	Bishop, 1979
Otariids					
Sea lion (<i>Zalophus californianus</i>)	Captive	HP	No*	France	Huet, 1882
		HP, IHC, TEM	Yes	Florida	Mense et al, 1992
		HP, IHC	Yes	Florida	Dubey et al, 2003b
Fur seal (<i>Callorhinus ursinus</i>)	Wild	HP	No	Alaska	Brown et al, 1974
Odontocete cetaceans					
Pilot whale (<i>Globicephala melana</i>)	Wild	HP	No	Newfoundland	Cowan, 1966
Sperm whale (<i>Physeter catodon</i>)	Wild	HP	Unknown	Unknown	Owen and Kaklaus, 1968
		HP	Unknown	Australia	Munday et al, 1978
Beluga (<i>Delphinapterus leucas</i>)	Wild	HP, M	No	Quebec	DeGuise et al, 1993
White-sided dolphin (<i>Langorhynchus acutus</i>)	Wild	HP, TEM	No	Massachusetts	Ewing et al, 2002
Striped dolphin (<i>Stenella coeruleoalba</i>)	Wild	HP	No	Oregon	Dailey and Stroud, 1978
		HP, IHC, TEM	Yes	Spain	Resendes et al, 2002b
Mysticete cetaceans					
Sei whale (<i>Balaenoptera borealis</i>)	Wild	HP, TEM	No	Japan	Akao, 1970

Continued

Table 40-1—cont'd

Protozoan and Marine Mammal Species	Wild or Captive	Tests for Detection	Parasite-Associated Disease?	Animal Location	Reference
<i>Sarcocystis</i> spp. and <i>S. canis</i>-like spp.—cont'd					
Mustelids					
Sea otter (<i>Enhydra lutris</i>)	Wild	HP, TEM, PCR	No	Washington	Dubey et al, 2003a
River otter (<i>Lutra lutra</i>)	Captive	HP, TEM	No	Norway, Sweden	Wahlstrom et al, 1999
Ursids					
Polar bear (<i>Ursus maritimus</i>)	Captive	HP, IHC, TEM	Yes	Alaska	Garner et al, 1997
<i>Neospora caninum</i>					
<i>N. caninum</i> infection has not been confirmed in marine mammals; the following are serologic surveys.					
Phocids					
Harbor seal (<i>Phoca vitulina</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Ringed seal (<i>Phoca hispida</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Bearded seal (<i>Erignathus barbatus</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Otariids					
Sea lion (<i>Zalophus californianus</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Walrus (<i>Odobenus rosmarus</i>)	Wild	S	No	Alaska	Dubey et al, 2003b
Odontocete cetaceans					
Bottlenose dolphin (<i>Tursiops truncatus</i>)	Wild, captive	S	No	California, Florida	Dubey et al, 2003b
	Wild	S	No	Solomon Islands	Omata et al, 2005
Killer whale (<i>Ocinus orca</i>)	Wild	S, PCR	No	Japan	Omata et al, 2006
Mustelids					
Sea otter (<i>Enhydra Lutris</i>)	Wild	S	No	California, Washington	Dubey et al, 2003b
		S (all seroneg)	No	California, Washington, Alaska	Miller and Conrad ⁵⁷
Unidentified apicomplexan parasite					
Phocids					
Harbor seal (<i>Phoca vitulina</i>)	Wild	HP, IHC, PCR, TEM	Yes	California	LaPointe et al, 2003

from the Pacific Coast of North America, suggesting high levels of environmental exposure to these potential pathogens.^{14,48,59}

Fatal infections by *S. neurona* are common in Pacific harbor seals (*Phoca vitulina richardsi*) and southern and northern sea otters from California, Washington, and Oregon.* This parasite is one of the more common causes of harbor seal mortality at the largest marine mammal rehabilitation facility in California, affecting primarily adult and subadult seals.^{12,13,45,59} In California, *S. neurona* is a major cause of wild sea otter mortality, causing severe meningoencephalomyelitis, lymphadenopathy, pneumonia, musculoskeletal and cardiovascular disease; this parasite appears to be increasing as a cause of sea otter death.^{42,43,48,56,60} A single wild cetacean from coastal California with possible *S. neurona* infection has also been recognized (see Table 40-1).

In contrast, *T. gondii*– or *S. neurona*–related mortality has not been reported from Alaskan or Russian (*Enhydra lutris lutris*) sea otters, although seropositive Alaskan otters have been detected.³⁰ Similarly, no sirenians or otariids have been reported with *S. neurona* infection, although they inhabit coastal areas where opossums reside in North and South America. Reports of asymptomatic *S. neurona* infections are absent from the marine mammal literature. This is undoubtedly a result of (1) underreporting of incidental cases and (2) difficulty distinguishing *S. neurona* tissue cysts from related, presumably less pathogenic, *Sarcocystis* species on histopathology.

Other *Sarcocystis* spp. Infecting Marine Mammals

Reports of *Sarcocystis* spp. infection in marine mammals span over 130 years and encompass more than 14 species, including otariids, phocids, cetaceans, mustelids, ursids, and sirenians (see Table 40-1). The first report of any tissue cyst-forming coccidial infection in a marine mammal was described by Huet³⁹ in 1882 from skeletal muscle of a sea lion that died in captivity in France (see Table 40-1). Huet attributed the death of this animal to lungworm infection, although intramuscular tissue cysts were grossly visible at necropsy. The only reported infection of a baleen whale by tissue cyst-forming coccidia is *Sarcocystis* spp. tissue cysts noted incidentally in skeletal muscle of a sei whale (*Balaenoptera borealis*) harvested for human consumption.¹

Little is known about the identity, host range, or definitive hosts for these parasites. However, some have been distinguished from *S. neurona* based on the disease presentation, parasite ultrastructure, immunohistochemistry, molecular characterization, and parasite distribution in host tissues.^{26,51,74,83} Reports of *Sarcocystis* spp. parasitism excluding *S. neurona* tend to fall into one of two major groups: (1) animals with tissue cysts (sarcocysts) noted incidentally in skeletal or cardiac muscle at necropsy, accompanied by variable, often minimal chronic infection, and (2) animals with severe necrotizing hepatitis associated with proliferation of intermediate parasite stages.

Muscle sarcocysts have been reported incidentally from sea otters, river otters, phocids, otariids, mysticetes, and odontocetes (see Table 40-1). Definitive speciation is not possible by conventional histopathology; with transmission electron microscopy (TEM); however, marked differences are often apparent, particularly in the structure of the cyst wall.²⁶ Given the large range of *Sarcocystis* spp. reported in terrestrial animals, it is likely that numerous species infect marine mammals, possibly including marine-adapted species. Limited molecular and ultrastructural characterization has suggested that these *Sarcocystis* spp. sarcocysts are distinct from *S. neurona*, but may be present concurrently with *S. neurona* in some animals, making accurate confirmation of infection at the light microscopic level a challenge.^{26,48}

Sarcocystis spp. parasites associated with fatal necrotizing hepatitis are reported from captive California sea lions (*Zalophus californianus*), a monk seal (*Monachus schauinslandi*), two polar bears (*Ursus maritimus*), and a wild striped dolphin (*Stenella coeruleoalba*) (see Table 40-1). In these animals, protozoal merozoites proliferate in the liver, causing severe hepatic damage. Some reports tentatively identify these parasites as *Sarcocystis canis*, although further characterization is needed.^{30,74,83}

Other Tissue Cyst-Forming Protozoa Infecting Marine Mammals

An unidentified protozoan was reported in 1994 from a Pacific harbor seal from Northern California.⁴⁶ Histopathology revealed necrotizing meningoencephalitis with intralesional protozoal tachyzoites. This parasite is antigenically distinct from *T. gondii*, *S. neurona*, and *Neospora caninum* and remains unidentified.

High seroprevalence of some marine mammals, especially cetaceans, for *N. caninum* has been reported,³⁰

*References 12, 29, 30, 42, 43, 45, 48, 49, 59, 60, 75, 78.

but no confirmed *N. caninum* infections have been reported in marine mammals. More than 500 necropsied California sea otters have been screened since 1998 with histopathology, immunohistochemistry (IHC), parasite isolation, and polymerase chain reaction (PCR); all have proved negative thus far for *N. caninum*,⁵⁷ although neosporosis is a significant problem in California dairy cattle. Canids are the definitive hosts for *N. caninum*. Rarely, southern sea otters with high antibody titers to *T. gondii* exhibit low, presumably cross-reactive titers to *N. caninum*, but they are negative for *N. caninum* on histopathology, IHC, and PCR. However, serologic cross-reactivity with *T. gondii* does not appear to be a factor in some cases. In one recent study, titers to *N. caninum* were detected in marine species that occurred in the absence of a positive titer for *T. gondii*.³⁰ This finding is intriguing and could be substantiated with confirmation of *N. caninum* infection in marine mammals in the future. Significant potential also exists for serologic cross-reactivity with other, as-yet unrecognized protozoa. Caution is advised when evaluating serologic tests in marine mammals; efforts to authenticate the results using more specific tests are warranted.

INDIRECT EFFECTS OF PROTOZOAL INFECTION AND DISEASE

In addition to death from meningoencephalitis and other systemic disease, potential indirect causes of mortality exist for *T. gondii*- and *S. neurona*-infected marine mammals, including increased susceptibility to trauma.^{6,42} Negative impacts of *T. gondii* brain infection on mentation and behavior are also reported in humans, laboratory animals, and experimentally infected gray seals.^{14,32} Reproductive impacts, such as fetal death or abortion from transplacental *T. gondii* infection, may prove to be important for some populations of marine mammals, particularly cetaceans.^{41,73} In addition, cardiovascular, pulmonary, lymphoreticular, and musculoskeletal pathology caused by *T. gondii* and *S. neurona* infection is reported from many species of marine and terrestrial animals. Protozoal-associated cardiac pathology may be challenging to distinguish from that caused by other agents because (1) affected animals often strand late in the course of the disease, when cardiac damage is extensive, and (2) more than one agent may be associated with cardiac pathology; for example, protozoal infection and domoic acid intoxication may affect animals concurrently.^{43,56}

ETIOLOGIC AGENT AND TRANSMISSION

Toxoplasma gondii

The genus *Toxoplasma* consists of a single species, *T. gondii*, with several distinct strains, or genotypes.^{14,63} *Toxoplasma gondii* is a single-celled protozoan parasite with a complex life cycle involving both definitive (oocyst-shedding) hosts and intermediate hosts that support the tissue cyst stage of the parasite.⁷⁷ Virtually any warm-blooded vertebrate, including humans, may serve as intermediate hosts. Thus far, only domestic and wild felids (cats) are known to serve as definitive hosts and support the sexual phase of the parasite's life cycle, producing millions of infective oocysts that are shed in the cat's feces (Figure 40-1). New hosts are infected through consumption of tissue cysts in muscle, brain, or other organs of intermediate hosts; by consumption of oocyst-contaminated paratenic hosts; or by accidental consumption of sporulated oocysts derived from the feces of infected cats in soil, in water, or on vegetation, or transplacentally.⁷⁷ Transplacental transmission is also well documented.⁷⁷ The interval between parasite ingestion by cats and oocyst shedding may be as short as 2 to 3 days when cats ingest *T. gondii* tissue cysts in the flesh of intermediate hosts.

Infected cats that are actively shedding oocysts typically exhibit no clinical abnormalities and may pass 100 million infective oocysts in their feces over a 10- to 14-day period.⁷⁷ Once present in the environment, oocysts become infective within 1 to 5 days and may remain viable for months to years under optimal conditions. Protozoal oocysts (*T. gondii* and *N. caninum*) and sporocysts (*S. neurona*) are resistant to most disinfectants but are susceptible to boiling water, formalin, and iodine. The infective dose may be as low as a single sporulated oocyst.

Intermediate hosts for *T. gondii* include all warm-blooded animals.⁷⁷ These hosts are infected through consumption of other infected intermediate hosts or oocysts. Once ingested by intermediate hosts, these parasites leave the intestinal tract and spread systemically as invasive, rapidly multiplying, intracytoplasmic tachyzoites. Occasionally these infections are fatal, especially for fetuses, immunosuppressed individuals, or highly susceptible animals, including Australian marsupials and New World monkeys. As specific host immunity is generated, conversion from rapidly multiplying tachyzoites to slower, less active bradyzoites occurs, and bradyzoite-filled tissue cysts develop, primarily in the brain, spinal cord, skeletal muscle, and myocardium

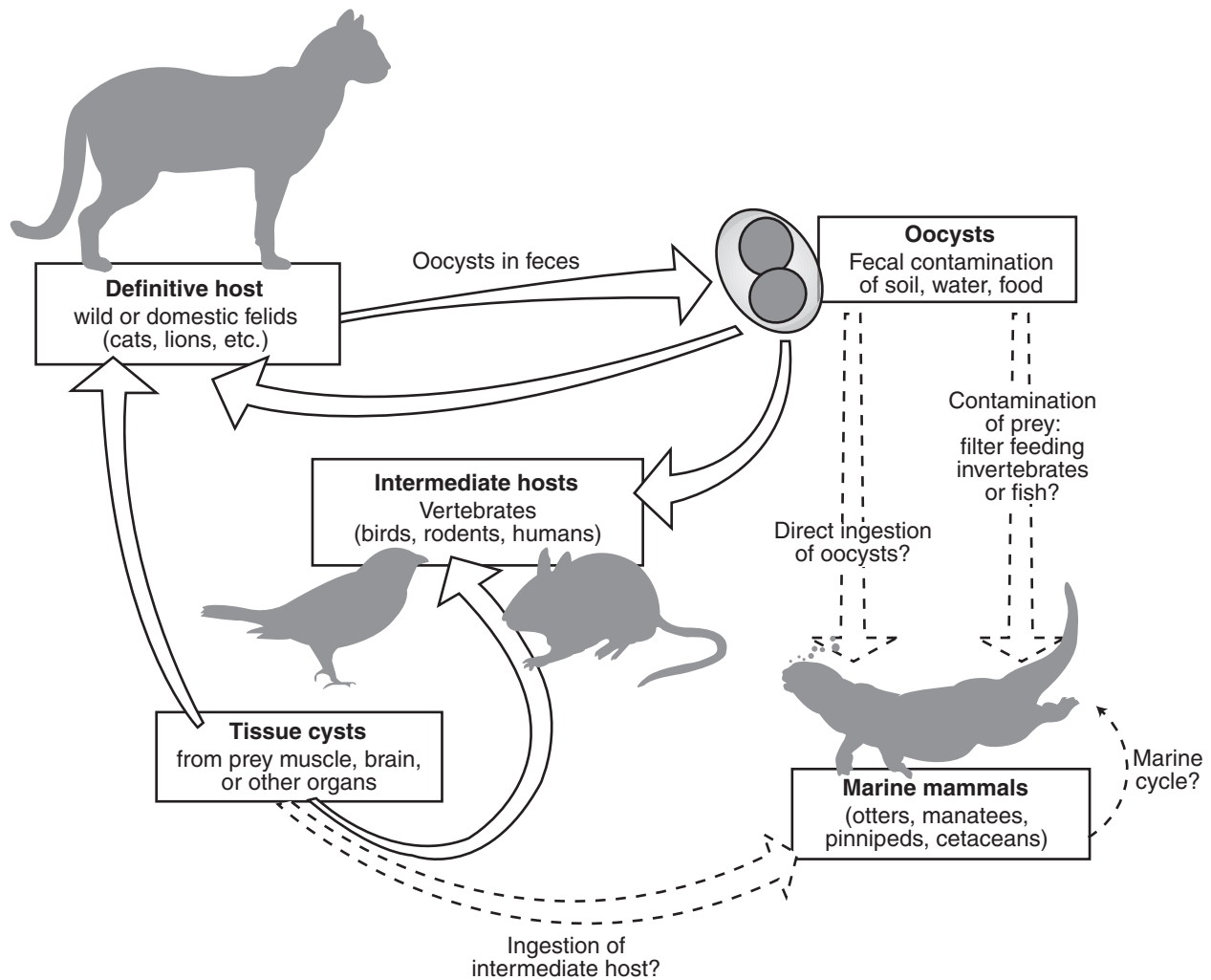


Fig 40-1 Life cycle of *Toxoplasma gondii* showing potential sources of marine mammal exposure (hatched lines).

(Figure 40-2). Once present, tissue cysts may persist for the life of the host, and infection becomes chronic, perhaps lifelong. Recrudescence of active infection with development of rapidly invasive tachyzoites may occur in chronically infected humans and animals when host immunity wanes.⁷⁷ For marine mammals, parasite recrudescence with concurrent disease may also be important, especially for *T. gondii*-infected animals; numerous reports exist in the literature of animals with distemper, sepsis, or other significant disease in addition to toxoplasmosis* (see Table 40-1).

Sarcocystis spp. and *S. neurona*

The genus *Sarcocystis* is large and diverse with many uncharacterized species. For well-described

species, the life cycles alternate between carnivores or omnivores, and herbivores (e.g., coyote-deer), with the carnivore/omnivore as definitive host and herbivores as intermediate hosts. In contrast to *Toxoplasma*, the genus *Sarcocystis* consists of numerous species, each utilizing a different range of definitive and intermediate hosts to complete their life cycle.²⁷ Little is known about the life cycles or host dynamics of *Sarcocystis* spp. infecting marine mammals, other than a putative link to opossums for *S. neurona*.³⁰

As noted earlier, *S. neurona* first gained wide recognition as the primary cause of EPM. The life cycle for this parasite is similar to *T. gondii*, but with some important differences. New World opossums (*Didelphis virginiana* and *D. albiventris*) are the only confirmed definitive hosts for *S. neurona*. As with cats, opossums appear to be asymptomatic hosts for *S. neurona* and may shed large numbers of sporocysts without clinical signs. However, unlike *T. gondii*, prolonged or repeated fecal shedding

*References 6, 8, 20, 21-23, 34, 40, 76, 81.

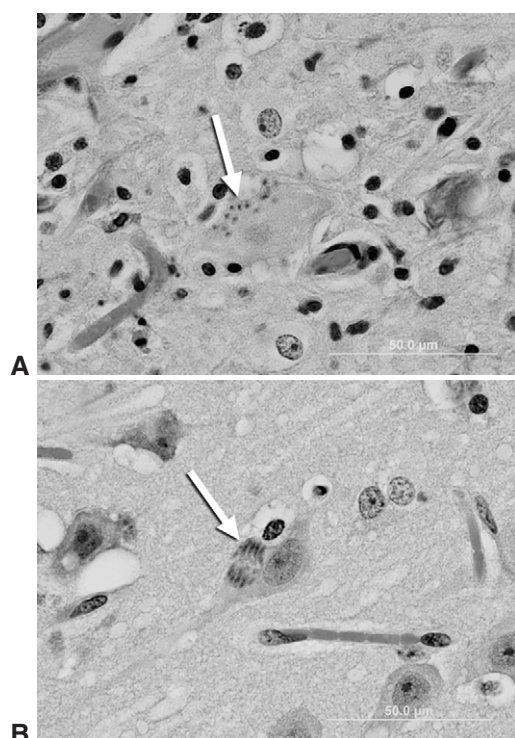


Fig 40-2 Comparison of *Toxoplasma gondii* tachyzoites and *Sarcocystis neurona* merozoites in infected neurons, sea otter cerebrum. **A**, High-magnification view of sea otter cerebrum. At the center of the photograph the cytoplasm of a neuron contains 12 or more short, stout, elliptic, brightly eosinophilic *T. gondii* tachyzoites (arrow). The tachyzoites are sometimes arranged in loose pairs or may have a more random, scattered appearance, as shown. **B**, Cerebrum from another sea otter at the same magnification. At the center of the photograph the cytoplasm of a neuron contains 10 or more long, slender, deeply basophilic *S. neurona* merozoites (arrow). These merozoites are often arranged in a circle or are aligned in groups, as shown. (Hematoxylin and eosin [H&E]-stained paraffin sections.) (See Color Plate 40-2.)

of *Sarcocystis* spp. by opossums has been demonstrated. The infective stage for *S. neurona* is a sporocyst that is immediately infective when defecated.^{24,30}

The intermediate host range for *S. neurona* includes a wide variety of birds and mammals, excluding humans.^{24,30} Invasive parasite stages resulting from asexual division of *S. neurona* are called *merozoites*, which penetrate host cells and proliferate to form *schizonts* and later tissue cysts. Sea otters and harbor seals are capable of serving as intermediate hosts, with tissue cyst formation in heart, skeletal muscle, and other tissues and proliferation of merozoites in the brain, lung, and other locations.* Unlike *T. gondii*, tissue cyst formation has not been reported in the central nervous system for *S. neurona*-infected animals.

Sarcocystis neurona also differs from *T. gondii* regarding the dominant pattern of asexual division occurring within host cells, a feature that may be used to distin-

guish these parasites microscopically and ultrastructurally (see Figure 40-2). Division of *T. gondii* occurs by *endodyogeny*, in which two new parasites are formed by lateral division within the cytoplasm of a “mother cell.”²⁴ Schizogony of *S. neurona* occurs by *endopolygeny*, in which “daughter” parasites (merozoites) bud off the surface of a mother cell, forming a distinctive, flowerlike radial arrangement called a *rosette-form schizont* (Figure 40-3).

Neospora caninum

Neospora caninum is closely related to *T. gondii* and shares some common features in its life cycle, including significant potential for vertical transmission.²⁴ The definitive hosts for *N. caninum* are canids, and numerous intermediate hosts have been identified, most notably dogs and cattle.²⁴ Neosporosis is a leading cause of abortion in dairy cattle worldwide.²⁴ Vertical transmission is a major route of parasite propagation, and infected cows and dogs may transmit the parasite to their offspring through successive pregnancies.

CLINICAL SIGNS

For many stranded marine mammals with protozoal disease, clinical data are not available because the animals were found dead or died soon after stranding. Incidental or mild *T. gondii* infections have been identified in phocids, otariids, mustelids, and cetaceans (see Table 40-1). Along the Pacific Coast of North America, reports of live-stranded marine mammals with severe *T. gondii*- or *S. neurona*-associated disease are common. Depending on the parasite involved, host age and immunocompetence, concurrent disease, and other factors, clinical signs may be absent or may consist of anorexia, depression, fever, central nervous system (CNS) disease, lymphadenopathy, icterus, abortion, stillbirth, and neonatal mortality.* Neurologic deficits are often the most prominent clinical abnormality and may include blindness, pupillary mydriasis, decreased mentation, sudden and episodic cessation of activity (e.g., eating, grooming), lack of aggression or increased aggression, unusual or repetitive stereotypic behavior, ambulatory and proprioceptive deficits, ataxia, paresis, paralysis, loss of bladder tone, obtundation, tremors, seizures, and coma.^{45,48,53,66,75} Tremors may worsen with stress, continued activity, or stimulation; may spread from one area (e.g., head, limb) to others, becoming generalized over time; and may be symmetric or asymmetric.

*References 29, 45, 48, 59, 60, 75.

*References 27, 29, 33, 45, 48, 49, 58, 74, 75, 83.

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Please refer to the printed publication.

Fig 40-3 Comparison of *Toxoplasma gondii* and *Sarcocystis neurona* parasite stages typically observed on histopathology and transmission electron microscopy. **A**, *T. gondii* tissue cyst from sea otter cerebrum. Note the thin outer cyst wall enclosing hundreds of tightly packed, banana-shaped bradyzoites (H&E-stained paraffin section, cyst diameter ~60 μm .) **B**, Transmission electron micrograph (TEM) of a developing *T. gondii* tissue cyst. At the center of the photograph, two daughter cells (DC) are forming by endodyogeny within a single "mother cell." Although endodyogeny is difficult to visualize on histopathology, parasites are sometimes arranged in loose pairs within the cytoplasm of infected cells before tissue cyst formation, providing a hint as to their identity as *T. gondii*. (Uranyl acetate and osmium-stained araldite thin sections, zoite length 2-3 μm .) **C**, *S. neurona* schizont from sea otter cerebrum. In contrast to *T. gondii*, *S. neurona* divides by endopolygeny, in which merozoites radially bud off the surface of a "mother cell," leaving behind a residual body. This form of division results in the formation of the characteristic flowerlike "rosette-form" schizonts typical of *S. neurona*, seen here. Note the radial arrangement of the tiny, deeply basophilic nuclei of the budding merozoites surrounding the central residual body. (H&E-stained paraffin section, schizont diameter ~10 μm .) **D**, TEM of rosette-form schizont of *S. neurona* from otter cerebrum. This is an ultrastructural view of the same process of endopolygeny shown in C. Note the radially arranged merozoites (M) budding off the surface of the residual body (R). (Uranyl acetate and osmium-stained araldite thin sections, schizont diameter ~10 μm .) (See Color Plate 40-3.) (Photomicrographs by Melissa A. Miller; A from Kreuder C, Miller MA, Jessup DA, et al: J Wildl Dis 39:495-509, 2003. Assistance with TEM preparation and interpretation by Robert Nordhausen and Bradd Barr of the California Animal Health and Food Safety Laboratory, Davis.)

Also common at necropsy of *T. gondii*- and *S. neurona*-infected marine mammals is the observation of diffuse, severe lymphadenopathy, sometimes with concurrent splenomegaly and nodular hyperplasia of splenic white pulp.^{30,42,56} A captive sea lion that died from toxoplasmosis exhibited severe lymphadenopathy, dysphagia, depression, and anorexia before death.³⁰ Reported musculoskeletal deficits include muscle tremors, myalgia, deficits in ambulation or proprioception, paresthesia, and paresis. Possible cardiac deficits include arrhythmias, tachycardia, pulse deficits, and clinical evidence of heart failure (e.g., ascites, generalized congestion, pulmonary edema, pleural effusion).^{43,58} Some animals exhibit severe *T. gondii*- or *S. neurona*-related interstitial pneumonia at necropsy, suggesting that protozoal infection should be considered for animals with diffuse respiratory disease.⁵⁶ Little clinical information is available for cetaceans or sirenians with protozoal disease because most are recovered dead or die soon after stranding. However, fetal and neonatal death caused by *T. gondii* appears prominent in cetaceans.^{41,73}

Differentiating between protozoal infections and other systemic disease, or between *T. gondii* and *S. neurona* infection is not feasible based purely on clinical signs; both parasites may cause severe disease and may be present concurrently in sick animals.^{14,49,59} Also, confirmation of infection is not equivalent to confirmation of disease; numerous reports exist of marine mammals with incidental *T. gondii* and *Sarcocystis* spp. infections (see Table 40-1).^{10,56} Careful screening for concurrent disease is also indicated.

Severe, necrotizing hepatitis caused by *Sarcocystis* parasites that appear distinct from *S. neurona* are described from polar bears, sea lions, a monk seal, and a striped dolphin, as discussed previously (see Table 40-1). Reported clinical signs included acute anorexia, lethargy, gastrointestinal (GI) illness, and icterus, often with a short course and fatal outcome.^{33,83}

In summary, protozoal infection should be considered for any marine mammal that strands alive with CNS, lymphoreticular (e.g., lymph nodes, thymus, spleen), musculoskeletal, cardiovascular, respiratory, hepatic, or reproductive abnormalities. In the living animal, confirmation of infection is based on demonstration of elevated or rising antibody titers or clinical response to long-term antiprotozoal therapy (see Clinical Therapy). Additional diagnostic procedures, such as muscle biopsy, electrocardiography (ECG), brain magnetic resonance imaging (MRI) or computed tomography (CT), and PCR from serum, muscle, or cerebrospinal fluid (CSF), may be attempted for valuable or rare individuals. Important differential diagnoses for neu-

rologic disease include hypoglycemia, hypothermia, hyperthermia, hyponatremia, hypernatremia, trauma, aberrant parasite migration (larva migrans), other infectious disease (e.g., viral or fungal infection), and exposure to natural or anthropogenic toxins such as domoic acid.⁸⁰ These agents cause illness that could be confused with systemic protozoal infection or may interact synergistically to produce clinical disease.

DIAGNOSIS

Clinical Pathology

For animals with *T. gondii* or *S. neurona* infection, diagnostic blood testing other than protozoal serology rarely is informative. Hypoglycemia and elevated muscle or liver enzymes are found in many sick animals, including those without protozoal disease. Testing urine, serum, tissues, or GI contents for biotoxin exposure may help exclude these agents as contributing factors.

Serology

Because of long-term, perhaps lifelong, persistence of tissue cysts, detection of positive antibody titers is suggestive of infection and not simply prior exposure to *T. gondii* or *S. neurona*. A positive test on serology, however, is not synonymous with protozoal disease, even in species at high risk, such as sea otters, although higher titers are often detected in clinically ill animals.⁵⁷ Significant potential exists for misinterpretation of test results, especially if the test has not been validated for the species in question. When possible, it is best to corroborate results from serologic testing with those from other diagnostic tests.

Serologic assays for apicomplexan parasites such as *T. gondii*, *S. neurona*, and *N. caninum* include three main types: agglutination tests, fluorescence-based tests, and enzyme-linked immunosorbent assay (ELISA). Commonly used agglutination tests for diagnosis of protozoal infection include the direct agglutination (DAT), modified agglutination (MAT), and latex agglutination (LAT) tests. These tests share three excellent attributes: (1) availability on a collaborative or commercial basis, (2) ease of use, and (3) ability to screen animals for which no species-specific antibodies are available. Agglutination tests are widely used to detect antibodies to *T. gondii* and *S. neurona* in marine mammals but have not been validated for these animals, instead relying on prior test validation in laboratory animals or pigs.

Other serologic tests used to detect protozoal infection in marine mammals include ELISAs and Western blots. ELISAs are efficient for rapid screening of multiple serum samples, but as with agglutination tests, may have problems with sensitivity and specificity when used to evaluate hemolyzed or debris-rich samples. Western blots have been used to confirm *S. neurona* infection in harbor seals⁴⁵ and may provide a fine degree of discrimination of serologic reactivity, but these tests are labor intensive and expensive.

Indirect fluorescent antibody tests (IFATs) are typically used to screen seals, sea lions, and sea otters for exposure to *T. gondii* and *S. neurona*, and an IFAT for *T. gondii* has been validated for sea otters.^{14,36,61} Because of the fluorescence-based reporting system, this assay is minimally susceptible to error associated with hemolysis or cellular debris.⁶¹ However, IFATs are labor intensive and more subjective regarding interpretation than ELISAs and agglutination tests, making rigorous standardization for test preparation and interpretation essential. However, suitable antisera and other reagents are increasingly available on a commercial and collaborative basis to detect protozoal immunoglobulin M (IgM) and IgG from serum of pinnipeds, mustelids, cetaceans, and other marine species.^{14,59,61}

Histopathology and Immunohistochemistry

Relying solely on hematoxylin and eosin (H&E)-stained tissues for case interpretation may result in under-recognition of the full impact of protozoal infection or disease; parasite-related damage may be focally severe and the causative lesion(s) easily missed in the tissue sampling process. Distinguishing between incidental parasitism and significant disease may also be a challenge because protozoal infections often present as a gradation from incidental to severe. Considering protozoal findings in the context of other diagnostic results and lesions will help. Animals that die after receiving antiprotozoal medications may not exhibit typical brain lesions because these medications cross the blood-brain barrier and may kill rapidly dividing parasite stages (e.g., tachyzoites, merozoites). However, IHC often reveals additional parasites in brain tissue, and tissue cysts (which are resistant to antiprotozoal therapy) may be found in the muscle, brain, and heart, even after long-term antiprotozoal therapy.⁵⁸

Immunohistochemical stains for *T. gondii*, *S. neurona*, *N. caninum*, and related parasites are available commercially and collaboratively (Figure 40-4; see also

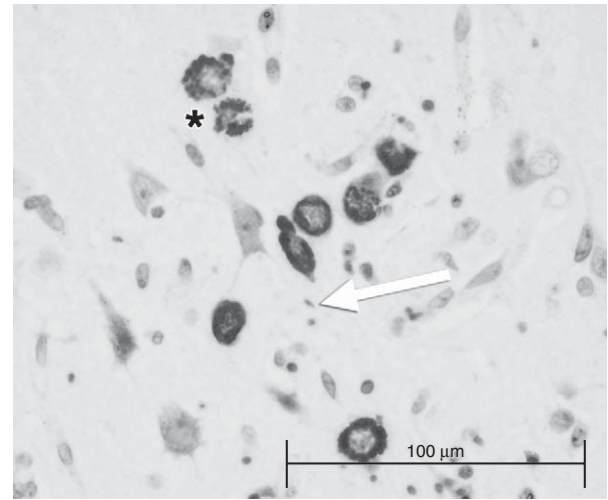


Fig 40-4 *Sarcocystis neurona* in sea otter cerebrum, visualized using immunohistochemical techniques, high-magnification view. Numerous *S. neurona* schizonts (*) and merozoites (arrow) are stained red-brown, making them easier to detect on the microscope. (Paraffin section stained using polyclonal antiserum raised to *S. neurona* and counterstained with hematoxylin.) (See Color Plate 40-4.)

Figure 40-3). Most IHC stains work well on formalin-fixed, paraffin-embedded tissues, but prolonged formalin fixation may lead to false-negative test results. These tests are useful both to help identify the parasites and to determine the spatial relationships between parasite profiles and areas of tissue inflammation and necrosis, thus helping to confirm or exclude a diagnosis of protozoal disease. However, paraffin histopathology and IHC cannot precisely differentiate between *S. neurona* and other *Sarcocystis* spp. tissue cysts.^{26,49} Also, IHC stains for *T. gondii* will label all extraintestinal tissue stages (e.g., tissue cysts, bradyzoites, tachyzoites), whereas antisera raised to *S. neurona* or *Sarcocystis* spp. strongly label merozoites and schizonts, but less reliable staining and cross-reactivity are reported for bradyzoites within tissue cysts.⁴ Thus, taxonomic differentiation of sarcocysts is best determined through TEM and PCR.

Parasite Isolation in Cell Culture and Mouse Bioassay

These specialized diagnostic techniques are available on a collaborative basis through universities and governmental agencies. Both techniques are time consuming and expensive, but may confirm infection and provide parasites for PCR, genotyping, reagent preparation, and research. Both techniques require collection of fresh, nonfrozen, sterile tissue from infected animals, including central nervous system, heart, and

skeletal muscle. At the laboratory, the tissue is often minced, partially digested with enzymes and layered over live, uninfected cells. Viable protozoa present in the tissue will infect the cultured cells and can then be maintained in the laboratory indefinitely. Alternatively, similar tissue preparations may be prepared for infection of laboratory mice.³⁰

Transmission Electron Microscopy

Transmission electron microscopy (TEM) may be used to confirm the presence of protozoa in the sample and to identify the protozoal genus and species (see Figures 40-2 and 40-3). A detailed description of ultrastructural characterization is beyond the scope of this chapter, but some basics are discussed in previous sections. The assistance of persons experienced in interpreting protozoal ultrastructure is recommended.

Sequencing and Genotyping

Commercial, university, and governmental laboratories may facilitate screening of tissues or other samples for the presence of protozoal deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), and parasite sequences are available for comparison in web-based databases such as GenBank. When possible, sequencing is recommended to ensure correct interpretation of amplified DNA or RNA bands and to avoid missing novel parasites. Recent technology facilitating discrimination of parasite subtypes (e.g., genotypes) may greatly aid investigations of relationships between protozoal infection and clinical outcome in marine species and may help trace environmental sources for infection.^{10,14,63} If a marine cycle for *T. gondii* or *S. neurona* exists, comparison of genotype frequencies between marine species and adjacent terrestrial animals could confirm their existence through discovery of genotypes that are unique to the marine environment.

PATHOLOGY

Gross Lesions

There are no pathognomonic gross lesions to confirm a diagnosis of protozoal infection by *T. gondii* or *S. neurona*; accurate diagnosis requires histopathology and other techniques. The most consistent gross findings in marine mammals infected with *T. gondii* or *S. neurona* are lymphadenopathy and splenomegaly.^{42,59,60,73}

Affected sea otters sometimes have orange-white mottling of the myocardium, serous pericardial effusion, pulmonary edema, interstitial pneumonia, symmetric or asymmetric muscle atrophy, an enlarged and urine-distended bladder, and multiorgan congestion, especially of the lungs, leptomeninges, and brain.^{42,56} Increased friability, pallor, or congestion, and gray to tan discoloration of brain tissue is noted rarely, along with flattening of gyri (brain swelling) and occipital or subtentorial brain herniation.⁵⁶

Prominent gross lesions may also include pulmonary emphysema,⁵³ emaciation,⁵⁴ hepatic necrosis and icterus,^{30,33,83} GI and adrenal hemorrhage,⁷³ and abortion, stillbirth, or neonatal mortality.^{41,73} Gross placentitis is not reported from marine mammals infected with *T. gondii*. An association between preexisting protozoal disease and traumatic death has been reported for southern sea otters and humpbacked dolphins.^{6,42} Behavioral impacts associated with experimental infection with *T. gondii* were also noted for gray seals.³²

Histopathology

Toxoplasma gondii

The most common microscopic lesion reported from marine mammals with toxoplasmosis is meningoencephalitis (see Table 40-1). Thin-walled, sometimes angular-edged tissue cysts are often observed on histopathology, supportive of chronic or recrudescent infections (see Figure 40-3). Tissue cysts are not reported to elicit an inflammatory response and are sometimes observed in areas of neuropil with no adjacent inflammatory infiltrate. When present, this infiltrate is typically arranged as large, discrete, inflammatory nodules dispersed randomly throughout the cerebrum and cerebellum. Individual nodules may contain central areas of necrosis, gliosis, cavitation, and dystrophic mineralization. Parasite profiles (e.g., tissue cysts and tachyzoites) are often concentrated at the lesion periphery, possibly reflecting a centrifugal or outward-spreading pattern of parasite proliferation (Figure 40-5). Immunohistochemistry sometimes reveals free or intracellular tachyzoites within affected areas, suggestive of recrudescence or chronic, low-grade parasite turnover. Smoldering *T. gondii* infections may be tolerated by the host until a critical portion or volume of neuropil is damaged, culminating in clinical disease or death. In most *T. gondii*-infected sea otters, low numbers of parasites are observed outside the CNS, suggesting that infection is subacute to chronic in the majority of cases. The discovery of protozoal tachyzoites

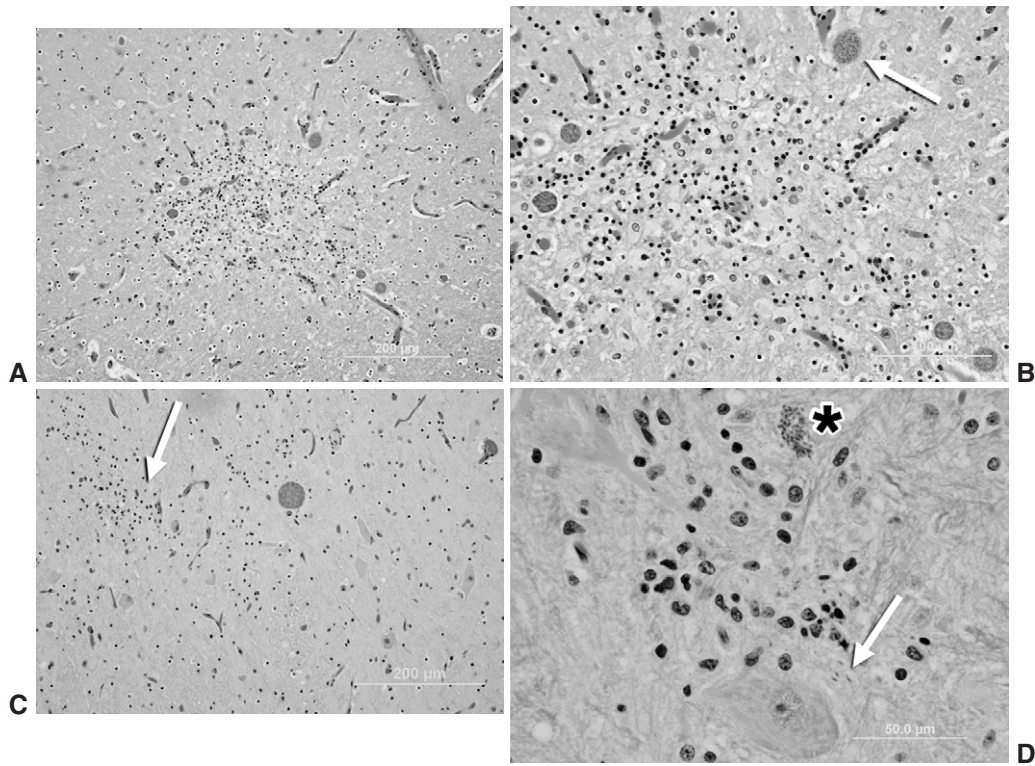


Fig 40-5 Protozoal meningoencephalitis: Comparison of lesions associated with *Toxoplasma gondii* and *Sarcocystis neurona* brain infections in sea otters. **A**, Cerebrum from sea otter with fatal *T. gondii*-associated meningoencephalitis. Brain infections with *T. gondii* are often characterized by detection of large, densely cellular inflammatory nodules composed primarily of small lymphocytes. A similar inflammatory infiltrate is often observed surrounding adjacent blood vessels and within the overlying meninges. **B**, Higher-magnification view of same area as in **A** showing development of *T. gondii* tissue cysts (arrow) at periphery of inflammatory lesion. This pattern of parasite proliferation is common in animals with *T. gondii*-associated meningoencephalitis. Tachyzoites are often difficult to see but may be apparent on immunohistochemistry. **C**, Cerebrum from sea otter with fatal *S. neurona*-associated meningoencephalitis, characterized by development of numerous small, loose clusters of mixed inflammatory cells (arrow). The inflammatory infiltrate is typically less dense and more pleocellular than for *T. gondii*-infected animals and includes lymphocytes, plasma cells, macrophages, and small numbers of neutrophils. Interestingly, although this otter died from *S. neurona*-associated meningoencephalitis, it was concurrently infected with *T. gondii*; a large, round, *T. gondii* tissue cyst is apparent near the top center of the photomicrograph. On histopathology the *T. gondii* infection appeared incidental, because these parasite profiles were rare and were associated with minimal inflammation. **D**, Higher-magnification view of inflammatory lesion from otter with fatal *S. neurona*-associated meningoencephalitis. The mixed character of the inflammatory infiltrate is easily appreciated at this magnification. A schizont is visible at the top center of the photomicrograph (*), and numerous tiny, deeply basophilic, banana-shaped merozoites are scattered throughout the inflamed tissue (arrow). (See Color Plate 40-5.)

in lymph nodes, lungs, liver, and spleen with few or no tissue cysts is more suggestive of recent exposure.

In *T. gondii*-infected otters, the inflammatory response is dominated by a monomorphic population of small lymphocytes with admixed macrophages and plasma cells, often with prominent perivascular cuffs surrounding adjacent blood vessels. Occasionally the infiltrate is located adjacent to the ventricular system or within the choroid plexus, indicative of local extension through CSF. A similar infiltrate is often seen in the spinal cord, although parasites are often sparse,

in contrast with *S. neurona*-infected otters. Viral, toxic, immune-mediated, and fungal diseases may incite a similar inflammatory infiltrate.

Lesions reported from other tissues of *T. gondii*-infected marine mammals include nonsuppurative myositis or myocarditis, myonecrosis, epicardial steatitis, interstitial pneumonia and edema, necrotizing adrenitis, gastroenteritis and lymphoid necrosis, affecting the lymph nodes, thymus and spleen.^{23,38,40-43,53,56} Parasite profiles are most commonly observed in the brain, heart, skeletal muscle, adrenal gland, lung, and

lymphoid organs. The lymph nodes and splenic white pulp of affected animals are often enlarged and reactive, with prominent cortical and paracortical expansion, follicular depletion and moderate sinus histiocytosis with variable hemosiderin deposition. Reports are sparse regarding lesions in the placenta of pregnant females, in part because this tissue is often not sampled for microscopic examination. However, *T. gondii*-infected fetuses or neonates may exhibit acute, severe disseminated toxoplasmosis^{41,73} (see Figure 40-5).

Sarcocystis neurona

The character and pattern of inflammation associated with *S. neurona* infection are somewhat different from *T. gondii*, thus providing a strong hint as to the protozoal species present, even when parasites are sparse (see Figure 40-5). The infiltrate associated with *S. neurona* brain infection in sea otters is typically more pleocellular, with a mix of lymphocytes, plasma cells, macrophages, and low numbers of neutrophils. For *S. neurona*-infected animals, small lymphocytes are often less prominent, and the infiltrate is often more diffuse and finely necrotizing, with some predilection for white matter tracts. Instead of a few large, widely spaced inflammatory nodules, the infiltrate associated with *S. neurona* is often arranged as hundreds to thousands of small, loose clusters of leukocytes (glial nodules), often associated with small centralized areas of tissue necrosis and edema (see Figure 40-5). Lesions are sometimes most severe in the cerebellum and brainstem, although parasites are observed throughout the brain and spinal cord on IHC (see Figure 40-4). Parasite profiles may be difficult to see on hematoxylin and eosin-stained sections, especially for otters or seals treated with antiprotozoal medications.

Identification of putative *S. neurona* tissue cysts or merozoites outside the CNS (e.g., muscles, heart, lungs, lymph nodes, spleen) is common for otters and seals with *S. neurona* infection. In contrast to *T. gondii* infection, *S. neurona* tissue cysts are not typically reported from the neuropil of infected animals. Pending confirmatory tests, detection of rosette-form schizonts in brain tissue on histopathology provides strong preliminary evidence for *S. neurona* infection (see Figure 40-3). Confirmation of *S. neurona* and *T. gondii* infection using IHC or other techniques is advised, even when "classical" parasite stages are visualized, because infection by unidentified protozoa is also reported.⁴⁶ In some cases, concurrent *T. gondii* and *S. neurona* infections contribute to severe meningoencephalitis⁵⁶ (see Figure 40-5). *Sarcocystis neurona*-associated transplacental transmission has not been reported.

Other Tissue Cyst-Forming Protozoa

Sarcocystis spp. tissue cysts may be observed in muscle or myocardium without evidence of significant pathology other than mild lymphocytic myositis* (see Table 40-1). In other marine mammals, intrahepatic proliferation of an unidentified *Sarcocystis* spp. resulted in fatal necrotizing hepatitis,^{30,33,51,74,83} although none of these animals was reported with meningoencephalitis. Finally, a harbor seal that died with meningoencephalitis in 1994 was found to be infected with an unidentified apicomplexan parasite.⁴⁶

CLINICAL THERAPY

A variety of antiprotozoal medications have been used in *T. gondii*-infected humans, including atovaquone, sulfonamides (e.g., trimethoprim-sulfamethoxazole [TMS]), azithromycin plus pyrimethamine, clindamycin with pyrimethamine and folinic acid, co-trimoxazole, doxycycline, minocycline, and minocycline plus pyrimethamine. Potential side effects of these medications include liver and skin disease, seizures, and leukopenia. Pyrimethamine, 0.5 mg/kg every 24 hours (q24h) for 2 days, then 0.25 mg/kg q24h for 30 days; TMS, 15 mg/kg orally (PO) q12h for 30 days; and clindamycin, 12.5 mg/kg intramuscularly (IM) q12h, have been used on a limited basis in otters and seals with suspected or confirmed protozoal disease but were thought to be unsuccessful in restoring normal clinical function.⁶⁶

Oral anticoccidial medications such as ponazuril (e.g., Marquis, Bayer Animal Health Corporation; 5 mg/kg PO q24h for 30-60 days) and diclazuril (1-10 mg/kg q24-48h) have been more widely used for antiprotozoal therapy in sea otters and harbor seals.⁶⁶ Ponazuril is licensed in the United States for treatment of EPM in horses infected by *S. neurona*; long-term treatment (≥ 28 days) is recommended by the manufacturer. This oral medication crosses the blood-brain barrier and kills nonencysted parasites (e.g., merozoites, tachyzoites). No negative side effects of ponazuril treatment have been reported in otters or seals to date.⁶⁶

Only anecdotal information is available to assess efficacy of diclazuril and ponazuril treatment in marine mammals.⁶⁶ In otters and seals treated for longer than 2 weeks in stranding facilities in California, ponazuril therapy was associated with partial resolution of neurologic disease in some cases and a decline in antibody

*References 1, 5, 7, 18, 19, 26, 31, 35, 39, 52, 82.

titers to *T. gondii* or *S. neurona*.⁶⁶ Distinguishing effects of antiprotozoal therapy from those caused by clearance of biotoxins (e.g., domoic acid) or resolution of concurrent disease complicates assessment of clinical efficacy. Cases of increased protozoal titers after initiating treatment have also been noted, possibly from rapid killing of parasites with elaboration of parasite antigens.⁶⁶ Slightly better clinical response is observed for otters infected with *S. neurona*, possibly because this parasite tends not to form medication-resistant tissue cysts in the CNS.

In otters and seals, medical intervention often is ineffective once neurologic signs are apparent. A high percentage of animals that exhibit CNS disease at stranding die during the early stages of antiprotozoal therapy, even though merozoites and tachyzoites appear to be partially or completely cleared from the CNS during treatment.⁵⁶ Possible explanations for the high mortality observed early in the course of antiprotozoal therapy include (1) systemic inflammatory or anaphylactic response to the rapid death of large numbers of parasites; (2) elaboration of soluble parasite antigens, toxic factors, or host cytokines in response to parasite death; (3) acute exacerbation of the already-significant inflammatory response; (4) cumulative brain damage that is too severe for animals to survive; and (5) affected animals had other, concurrent disease in addition to protozoal disease. Tissue cysts are unaffected by ponazuril and other antiprotozoal medications, so posttreatment recrudescence is also a concern. Suspected recrudescence of *T. gondii* was observed clinically and histologically in two sea otters that restranded after seemingly successful long-term antiprotozoal therapy and release.⁵⁸

PREVENTION

For captive marine mammals, prevention of exposure to *T. gondii* and *S. neurona* should include efforts to exclude the definitive and intermediate hosts from enclosures, food preparation areas, and water sources. Birds common to outdoor exhibits, such as gulls, may serve as a source of *T. gondii* and *S. neurona* infection if caught and consumed by exhibit animals. However, birds and rodents will not shed *T. gondii* or *S. neurona* oocysts and sporocysts in their feces. Standard municipal water treatment practices, such as chlorination, will not completely neutralize oocysts or sporocysts.^{10,14,62} For captive animals fed live prey, obtaining these food items from pristine areas or allowing time for postcollection depuration in clean water may decrease the risk of prey contamination through filter-feeding activity. A simpler practice is to feed frozen-thawed

food items; freezing at 20° C for several days will kill tissue cysts, oocysts, and sporocysts. No vaccines are available for prophylactic treatment of marine mammals.

Current information on the life cycle and epidemiology of *T. gondii* for coastal California suggests that terrestrial hosts are the reservoir for infection of wild marine mammals. Accordingly, prevention and control may require major changes in the way that wastewater and feces are handled, processed, and discharged. Elimination of all potential definitive hosts is neither achievable nor desirable; humane control of introduced and feral animal populations, combined with conscientious disposal of pet waste in approved sanitary landfill facilities, might help. A recent survey of three small coastal California communities revealed that outdoor pet and feral cats deposited at least 100 metric tons of feces per year within a 2-mile radius of a high-risk area for *T. gondii* infection and disease for sea otters.¹⁷ A problem of this magnitude may be addressed only through global shifts in thinking and action. Further investigation of an endemic or adapted marine cycle is also needed.

ZOONOTIC POTENTIAL

The high frequency with which *T. gondii* infections are observed within some marine mammal populations demands that care be taken by persons handling unfixed and unfrozen tissues collected at necropsy, especially brain and spinal cord. Women who are pregnant or trying to become pregnant and persons with immune system abnormalities should avoid contact with marine mammal tissues and fluids. For routine necropsy and sample processing, serologic testing for exposure to *T. gondii* is advisable before beginning work and at regular intervals thereafter. Live parasites have been recovered from CSF, skeletal muscle, and myocardium of marine mammals, demonstrating that the risk for parasite exposure is not limited to brain tissue. Live parasites have also been isolated from tissues of significantly decomposed animals.^{57,60} Care should be taken to avoid cuts and needlesticks when performing necropsies, and potential exposures should be reported. Consultation with a physician experienced in infectious disease is recommended for potential exposures and serologic monitoring or antiprotozoal therapy may be considered, depending on the situation. Similarly, production of aerosols should be minimized, especially when opening the skull and vertebral column. Marine mammals do not appear to be definitive hosts for *T. gondii* or *S. neurona*, so passage of these parasites in the feces is not a significant concern.

However, marine mammals may shed oocysts or cysts of other zoonotic protozoa, such as *Cryptosporidium* and *Giardia*, in their feces. Unlike *T. gondii*, *S. neurona* has not been reported as a human pathogen.

POTENTIAL LAND-SEA CONNECTIONS AND ENVIRONMENTAL PATTERNS

The existence of a marine cycle for *T. gondii* and *S. neurona* and the ability of fish or invertebrate prey to serve as true intermediate hosts for these parasites have not been fully investigated. Although *T. gondii* and *S. neurona* infections are common in some marine mammal populations (see Table 40-1), evidence for intraspecific transmission other than vertical transmission is sparse. Most infections probably result from environmental exposure to oocysts or sporocysts shed in the feces of terrestrial definitive hosts (e.g., cats, opossums) and not through contact with infected conspecifics, as occurs with most other marine mammal pathogens. The high frequency of protozoal infection in some marine mammals suggests that environmental contamination with these parasites is extensive wherever potential definitive hosts are present. Once shed, oocysts or sporocysts may contaminate water or may be taken up by filter-feeders or detritivores. The importance of surface water runoff in the dissemination of protozoa to humans and marine mammals is increasingly recognized.^{14,62,77} Oocyst contamination of marine bivalves has been demonstrated experimentally, and once taken up by the invertebrate, these oocysts may remain infectious for several days.^{2,47} Factors that could influence oocyst infectivity, distribution, and survival in the marine environment include water temperature, salinity, currents, sediment type, and the range of planktonic and macroinvertebrate infauna.

However, marine mammals that do not consume invertebrates also become infected with *T. gondii* and *S. neurona* (see Table 40-1), suggesting that other efficient routes for exposure exist. Marine mammals rarely consume recognized intermediate hosts for *T. gondii* and *S. neurona*, such as rodents and birds; Manatees are herbivores but drink fresh water from surface drainage pipes, a potential source of oocyst exposure.⁸ Oocyst uptake by plankton, with subsequent predation by higher-trophic-level animals, including fish, could be one means by which piscivorous marine mammals could become infected. This means of oocyst uptake would enable *T. gondii* and *S. neurona* to utilize new intermediate hosts and to disseminate over a broader geographic area.

Studies in California have revealed specific risk factors for *T. gondii* exposure and disease in otters, including older age and male gender.^{42,43,62} In addition, coastal locations were identified where animals had a greater risk for protozoal infection and disease.^{14,42,62} Characteristics shared by high-risk areas on the central California coast include proximity to high-outflow streams and rivers and large, enclosed bays with limited tidal flushing action.^{14,42,62} In addition, both high-risk sites are located near large coastal power-generation facilities discharging heated water, although no causal link has been established. High-risk areas for marine mammal exposure to *T. gondii*, *S. neurona*, or other pathogenic protozoa undoubtedly exist elsewhere around the world.

Seasonal variation in *S. neurona* mortality is noted for sea otters, with more animals dying in the spring and summer months.⁴² This may result from both environmental and biologic factors; winter and spring are the periods of maximal rainfall along the central coast of California, when high numbers of sporocysts could be flushed downstream, placing marine mammals at higher risk. In addition, both otters and opossums reproduce during this period, placing high numbers of young, naive opossums and susceptible otters in close proximity.

Vertical (e.g., transplacental or transmammary) transmission is another potential route for protozoal parasites to gain access to new hosts and to propagate infection. In terrestrial animals, vertical transfer of *T. gondii* and *N. caninum* is well documented, and transplacental transmission of *T. gondii* is a major health concern for humans, associated with fetal abortion, hydrocephalus, mental retardation, blindness, and death.⁷⁷ Similar fetal impacts resulting from maternal infection with *T. gondii* have been documented in marine mammals,^{11,41,56,73} and it is likely that additional cases in marine mammals are missed because of fetal death, resorption in utero, or fetal loss at sea. Transplacental infection with *S. neurona* has not been reported. The role of vertical transmission as the primary means of propagating *T. gondii* infection in sea otters is not supported by data from long-term serologic screening and in vitro parasite isolation from brains of more than 330 freshly dead otters of all age classes.¹⁴

Several studies have reported correlations between significant protozoal disease in marine mammals and immunosuppressive factors, including morbilliviruses, anthropogenic pollutants, and bacterial sepsis.* However, concurrent immunosuppressive processes are not

*References 20, 21, 23, 34, 37, 76, 81.

always identified, and other factors, such as high environmental exposure, enhanced parasite infectivity or pathogenicity, new host-parasite interactions, and genetic factors, may also contribute to the high apparent susceptibility of some marine mammal species to *T. gondii* and *S. neurona*.⁶³ Given the complexity of the natural world, several factors are likely to be influencing the frequency and severity of protozoal infections in marine species.

FUTURE DIRECTIONS

Numerous studies are now in progress to better understand potential mechanisms of land-sea transfer of protozoal parasites, to explore marine mammal host ranges, and to determine methods for more effective detection and inactivation of oocysts in drinking water, wastewater, and prey species. New molecular techniques have revealed that the range of *T. gondii* genotypes present around the world are much more diverse than previously imagined. Focused studies of the various environmental niches occupied by various protozoal genotypes or strains will provide important clues as to their origins, population dynamics, and pathogenicity. Finally, the development of novel antiprotozoal therapies may help to more effectively treat marine mammals that strand with protozoal disease. Efforts to better understand these marine mammal protozoal infections may also greatly benefit humans, because we share the same coastal waters and consume the same foods as our marine counterparts.

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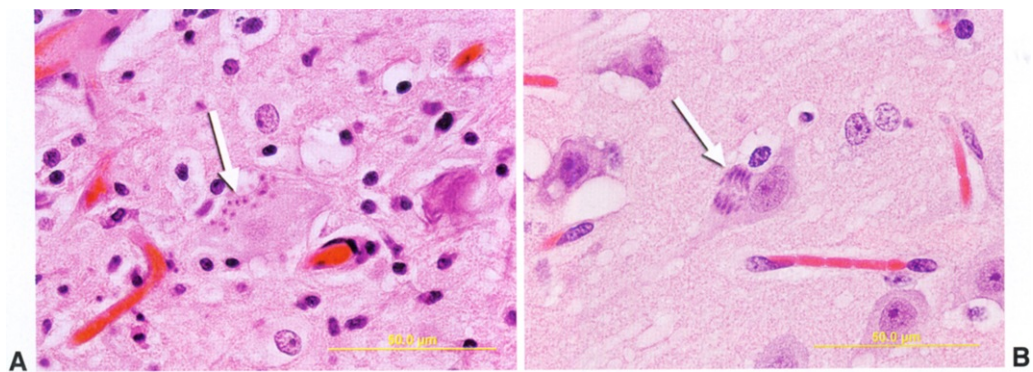
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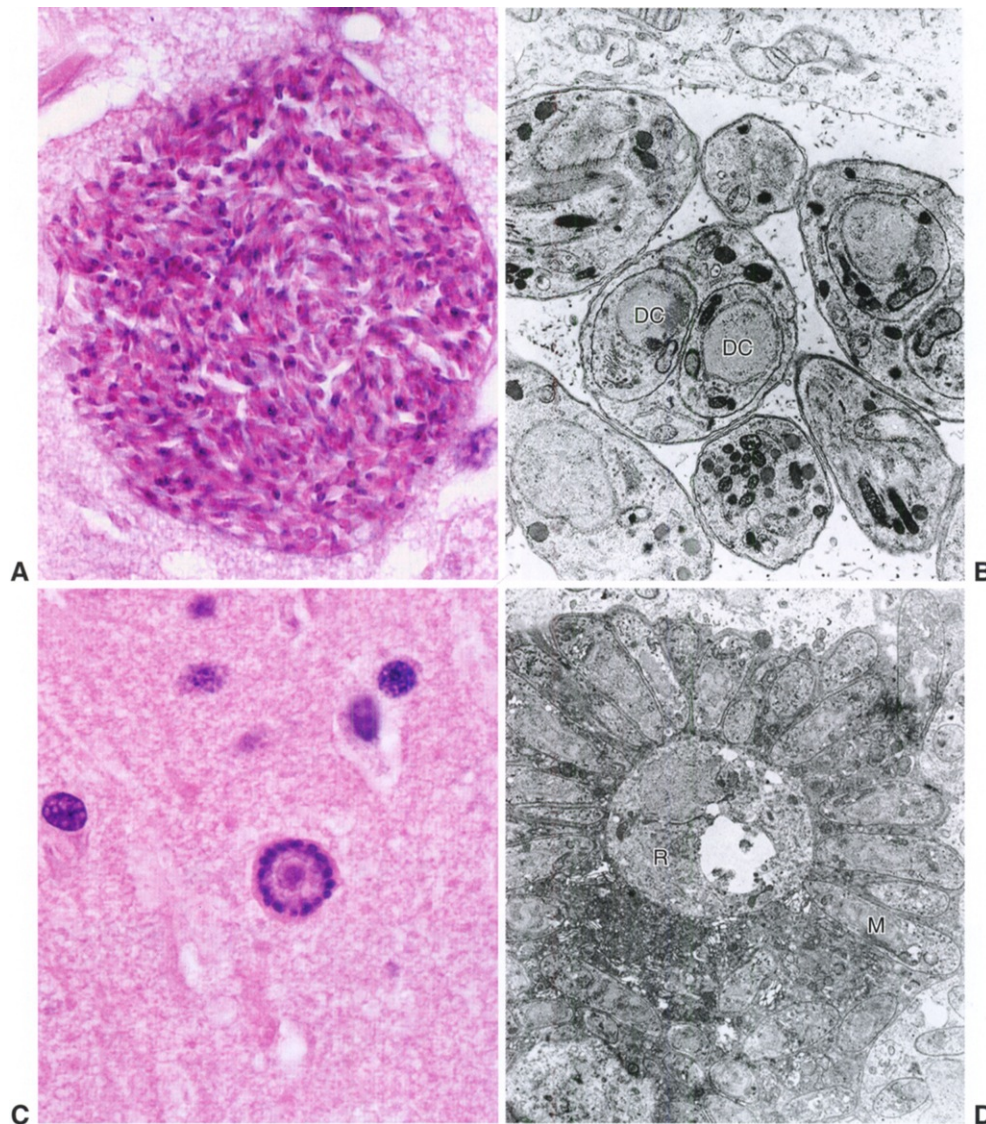
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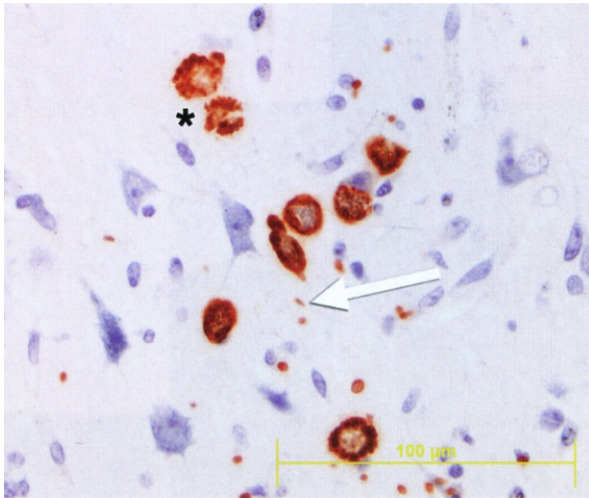
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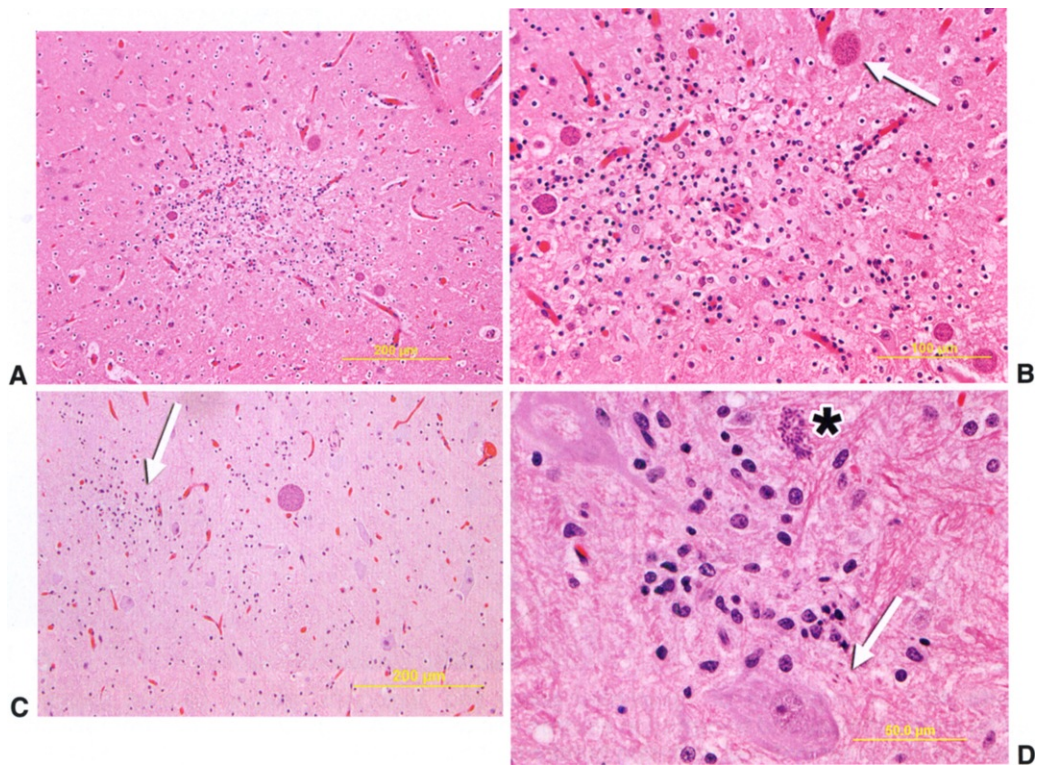
Color Plate 40-2 Comparison of *Toxoplasma gondii* tachyzoites and *Sarcocystis neurona* merozoites in infected neurons, sea otter cerebrum. **A**, High-magnification view of sea otter cerebrum. At the center of the photograph the cytoplasm of a neuron contains 12 or more short, stout, elliptic, brightly eosinophilic *T. gondii* tachyzoites (arrow). The tachyzoites are sometimes arranged in loose pairs or may have a more random, scattered appearance, as shown. **B**, Cerebrum from another sea otter at the same magnification. At the center of the photograph the cytoplasm of a neuron contains 10 or more long, slender, deeply basophilic *S. neurona* merozoites (arrow). These merozoites are often arranged in a circle or are aligned in groups, as shown. (Hematoxylin and eosin [H&E]–stained paraffin sections.) (For text mention, see Chapter 40, p. 328.)



Color Plate 40-3 Comparison of *Toxoplasma gondii* and *Sarcocystis neurona* parasite stages typically observed on histopathology and transmission electron microscopy. **A**, *T. gondii* tissue cyst from sea otter cerebrum. Note the thin outer cyst wall enclosing hundreds of tightly packed, banana-shaped bradyzoites (H&E-stained paraffin section, cyst diameter ~60 μm .) **B**, Transmission electron micrograph (TEM) of a developing *T. gondii* tissue cyst. At the center of the photograph, two daughter cells (DC) are forming by endodyogeny within a single "mother cell." Although endodyogeny is difficult to visualize on histopathology, parasites are sometimes arranged in loose pairs within the cytoplasm of infected cells before tissue cyst formation, providing a hint as to their identity as *T. gondii*. (Uranyl acetate and osmium-stained araldite thin sections, zoite length 2-3 μm .) **C**, *S. neurona* schizont from sea otter cerebrum. In contrast to *T. gondii*, *S. neurona* divides by endopolygony, in which merozoites radially bud off the surface of a "mother cell," leaving behind a residual body. This form of division results in the formation of the characteristic flowerlike "rosette-form" schizonts typical of *S. neurona*, seen here. Note the radial arrangement of the tiny, deeply basophilic nuclei of the budding merozoites surrounding the central, light-purple residual body. (H&E-stained paraffin section, schizont diameter ~10 μm .) **D**, TEM of rosette-form schizont of *S. neurona* from otter cerebrum. This is an excellent ultrastructural view of the process of endopolygony shown in C. Note the radially arranged merozoites (M) budding off the surface of the residual body (R). (Uranyl acetate and osmium-stained araldite thin sections, schizont diameter ~10 μm .) (For text mention, see Chapter 40, p. 329.) (Photomicrographs by Melissa A. Miller; A from Kreuder C, Miller MA, Jessup DA, et al: J Wildl Dis 39:495-509, 2003. Assistance with TEM preparation and interpretation by Robert Nordhausen and Bradd Barr of the California Animal Health and Food Safety Laboratory, Davis.)



Color Plate 40-4 *Sarcocystis neurona* in sea otter cerebrum, visualized using immunohistochemical techniques, high-magnification view. Numerous *S. neurona* schizonts (*) and merozoites (arrow) are stained bright red-brown, making them easier to detect on the microscope. (Paraffin section stained using polyclonal antiserum raised to *S. neurona* and counterstained with hematoxylin.) (For text mention, see Chapter 40, p. 331.)



Color Plate 40-5 Protozoal meningoencephalitis: Comparison of lesions associated with *Toxoplasma gondii* and *Sarcocystis neurona* brain infections in sea otters. **A**, Cerebrum from sea otter with fatal *T. gondii*-associated meningoencephalitis. Brain infections with *T. gondii* are often characterized by detection of large, densely cellular inflammatory nodules composed primarily of small lymphocytes. A similar inflammatory infiltrate is often observed surrounding adjacent blood vessels and within the overlying meninges. **B**, Higher-magnification view of same area as in **A** showing development of *T. gondii* tissue cysts (arrow) at periphery of inflammatory lesion. This pattern of parasite proliferation is common in animals with *T. gondii*-associated meningoencephalitis. Tachyzoites are often difficult to see but may be apparent on immunohistochemistry. **C**, Cerebrum from sea otter with fatal *S. neurona*-associated meningoencephalitis, characterized by development of numerous small, loose clusters of mixed inflammatory cells (arrow). The inflammatory infiltrate is typically less dense and more pleocellular than for *T. gondii*-infected animals and includes lymphocytes, plasma cells, macrophages, and small numbers of neutrophils. Interestingly, although this otter died from *S. neurona*-associated meningoencephalitis, it was concurrently infected with *T. gondii*; a large, round, *T. gondii* tissue cyst is apparent near the top center of the photomicrograph. On histopathology the *T. gondii* infection appeared incidental, because these parasite profiles were rare and were associated with minimal inflammation. **D**, Higher-magnification view of inflammatory lesion from otter with fatal *S. neurona*-associated meningoencephalitis. The mixed character of the inflammatory infiltrate is easily appreciated at this magnification. A schizont is visible at the top center of the photomicrograph (*), and numerous tiny, deeply basophilic, banana-shaped merozoites are scattered throughout the inflamed tissue (arrow). (For text mention, see Chapter 40, p. 334.)

CHAPTER 41

Algal Bloom Toxicity in Marine Animals

LINDA J. LOWENSTINE

Harmful algal blooms (HABs) are becoming more frequent along all coasts of the United States and throughout the world^{1,2} (<http://www.whoi.edu/redtide>). The microalgal species within these blooms, including diatoms and dinoflagellates, produce a variety of marine biotoxins that may be harmful to marine organisms and people. Although marine organisms have likely coexisted with algal blooms for eons, the increased magnitude and severity of exposure to HABs and the acute and often fatal nature of the intoxications seem to have led to minimum evolutionary adaptation. Thus, all taxa of marine organisms, from invertebrates to marine mammals, have died from the algal marine biotoxins. Massive “fish kills” and unusual mortality events involving large species, such as manatees, sea lions, whales, small cetaceans, and giant squid, have been particularly remarkable.

Several well-recognized intoxications are named for the symptoms experienced by humans, who are largely exposed through the consumption of filter-feeding shellfish containing the marine biotoxins (Table 41-1).²⁵ Briefly, these are *paralytic shellfish poisoning* (PSP) caused by saxitoxins; *neurotoxic shellfish poisoning* (NSP) resulting from brevetoxins; *amnesic shellfish poisoning* (ASP) from domoic acid; *diarrhetic shellfish poisoning* (DSP) from okadaic acid, yessotoxin, and other toxins; *azaspiracid shellfish poisoning* (AZP); and *ciguatera fish poisoning* (CFP, ciguatera) caused by ciguatera toxin and maitotoxin. Except in the case of ciguatera, shellfish are the usual vectors, although increasing evidence suggests that crustaceans and finfish may also be important in tropic transfer of all the algal biotoxins.^{10,36,43}

Of these intoxications, *brevetoxicosis* (NSP) has had the most impact on wildlife historically and currently, especially in Florida. Domoic acid intoxication is increasingly recognized as a cause of pinniped, otter, seabird, and possibly cetacean mortality on the U.S. West Coast, but blooms of *Pseudonitzschia* spp. are not

accompanied by the fish kills associated with red-tide blooms of *Karenia brevis*—secreting brevetoxins. *Saxitoxinosis* has been associated with deaths of marine birds and mammals. Ciguatera has been implicated in rare cases of pinniped mortality. The diarrhetic shellfish toxins and those of the azaspiracid group to date have not been associated with wildlife mortality. These latter two conditions are not covered in this chapter, except to mention that experimental work suggests that they may have an effect on fish embryos.

Although marine toxins are most frequently a threat to free-living marine organisms, the potential for contamination of natural seawater used in exhibits or enclosures and food items fed to aquatic animals managed in captivity is real.⁴⁴ Thus it is important for both zoo and wildlife veterinarians and pathologists to be familiar with the diagnosis, detection, and treatment of marine biotoxins in marine organisms. This chapter focuses on these aspects; for more detail about HABs, marine biotoxins, and experimental work in laboratory animals, consult review articles, government bulletins, and websites (<http://www.rsmas.miami.edu/groups/niehs>).*

MARINE TOXINS: TARGET TISSUES AND METHODS OF INTOXICATION

Brevetoxins are produced by *Karenia brevis* (formerly *Gymnodinium breve*, *Ptychodiscus breve*) and a few other microalgae.⁵⁷ Brevetoxin is actually a complex of several lipid-soluble polyether neurotoxins, designated PbTx 1 to 10, that open sodium-gated channels and cause influx of Na⁺ into cells. The toxins act on both the voluntary and the autonomic nervous system. Hemolytic toxins are also produced. Intoxication is by ingestion of contaminated prey or marine vegetation

*References 23, 25, 26, 51, 54, 57, 61.

Table 41-1

Major Microalgal Intoxications in Humans and Wildlife Species Affected

Toxin and Clinical Syndrome	Wildlife Species Affected (Vectors and/or Victims)	Location
Saxitoxins Paralytic shellfish poisoning (PSP)	Seabirds, whales, sea otters Fish: herring, salmon, menhaden, sand lance, mackerel, puffer fish Mussels, surfclams, softshell clams, sea scallops, butterclams, ocean quahogs, oysters, gastropods, lobsters, crabs, and other benthic invertebrates Squid and zooplankton	North Atlantic, Northeastern U.S., Pacific Northwest and Alaska, Europe, Japan, Australasia
Brevetoxins Neurotoxic shellfish poisoning (NSP)	Sea birds, manatees, dolphins Fish, sea turtles Bay scallops, surf clams, oysters, southern quahogs, coquinas Tunicates, sponges, coral	Subtropical regions: Gulf of Mexico, southern U.S. Atlantic coast
Domoic acid (DA) Amnesic shellfish poisoning (ASP)	Numerous bivalves, squid, crustaceans, fish, seabirds Otariid seals, sea otters Humpback and blue whales	Eastern Canada, west coasts of U.S. and Mexico, Gulf of California
Ciguatoxin, maitotoxin Ciguatera fish poisoning (CFP)	Fish: grouper, snapper, mackerel, jack, barracuda, parrot fish, tang, goat fish, and other finfish Gastropods	Caribbean Hawaii, South Pacific
Okadaic acid, dinophysistoxins, pectenotoxins, yessotoxin Diarrhetic shellfish poisoning (DSP)	Fish and shellfish as vectors, but no "fish kills" Experimental fish embryo death and deformities	Japan, Europe, Chile, Thailand, Canada (Nova Scotia), Australia, New Zealand
Azaspiracid toxins Azaspiracid shellfish poisoning (AZP)*	Shellfish vectors: mussels, oysters Incompletely studied	Ireland Not yet in North American waters

*Gastrointestinal disease.

or through inhalation of toxins aerosolized during a bloom. Minimum effective concentration in food for humans is suspected to be about 78 to 120 µg/mg. Clinical signs of NSP caused by ingestion include diarrhea with abdominal pain, myalgia, ataxia, and temperature reversal (cold objects feel hot), and signs caused by inhalation include lacrimation, rhinorrhea, coughing, and potentiation of asthma.¹⁶

Saxitoxins (STX) are produced by dinoflagellates such as *Alexandrium* spp., *Gymnodinium catenatum*, and *Pyrodinium bahamense*.⁵⁷ These toxins cause gastrointestinal (GI) signs and a variety of neurologic signs. Saxitoxins block sodium channels, preventing signal transmission along nerves. A dose of 1 to 4 mg of toxin is lethal in humans. Clinical signs of PSP include tingling and numbness beginning around the mouth, limb weakness, headache, nausea, and vomiting. Death may occur as acutely as within 4 hours of ingestion from respiratory paralysis.¹⁹

Domoic acid (DA) is a neuroexcitatory amino acid analog of L-glutamate produced by diatoms of the

genus *Pseudonitzschia*, along with some macroalgae.^{26,57} DA binds to glutamate receptors in the central nervous system (CNS), causing sustained membrane depolarization and leading to neuronal excitotoxic death. Elevation of endogenous glutamate potentiates the process. Neurons in the limbic system, including the hippocampus, are most at risk. Vomiting, diarrhea, disorientation, memory loss, seizures, coma, and death have been reported in humans affected by ASP. Minimum effective concentration in ingested seafood for humans is suspected to be about 60 mg for GI signs and 270 mg for neurologic signs. Oral doses of 5 and 10 mg/kg in monkeys cause gagging and vomiting.⁵⁵

Ciguatoxin and *maitotoxin* are the agents of CFP.^{54,57} These toxins are ingested in fish that have eaten the toxin-producing organisms *Gambierdiscus toxicus*, *Procerentrum* spp., *Ostreopsis* spp., *Coolia monotis*, *Thecadinium* spp., and *Amphidinium carterae*. The toxins are highly potent, and ingestion of as little as 0.1 ng will cause clinical signs in humans. Serious, but not usually fatal, the clinical signs of ciguatera in humans include

GI, neurologic, and cardiac effects such as arrhythmias and heart block. Neurocutaneous and systemic forms of illness may persist over a long time frame and may mimic multiple sclerosis or chronic fatigue syndrome.⁵⁴

Invertebrates

Invertebrate “shellfish,” principally bivalves and crustaceans, are important vectors for marine biotoxins, transferring the toxins to higher trophic levels. Some of the toxins (e.g., DA) are fairly rapidly excreted (*depurated*) by the invertebrates, whereas others (e.g., brevetoxins, saxitoxins) are lipophilic and may accumulate in tissues.

Bivalve and gastropod molluscs and octopi seem resistant to the effects of saxitoxin, and some accumulate high levels of the toxin without adverse effects.^{39,45} Some bivalves show adaptive aversion to toxic dinoflagellates.

Brevetoxins may be toxic to invertebrates. Loss of muscle control and decreased righting response are seen in crown conch (*Melongema corona*) and lettered olive (*Oliva sayana*), in which reported mortality rates varied from 55.5% to 69% (Florida Fish and Wildlife Institute, www.research.myfwc.com). No specific gross or histologic lesions could be attributed to the intoxication. Other invertebrates developing intoxication during Florida red-tide events caused by *K. brevis* include horseshoe crabs and jellyfish.⁵⁷ Deaths of starfish have resulted from brevetoxin-like compounds secreted by *Heterostigma* spp. marine flagellates.⁴⁴

Bivalves act as vectors of DA, and crabs such as the common sand crab also seem to uptake DA readily and may be vectors and useful monitors for the presence of DA in coastal environments.^{10,15} Effects of DA on bivalves have not been reported. A die-off of Humboldt squid (*Dosidicus gigas*) off the coast of California in 2005 was postulated to result from DA intoxication, and high DA levels have been found in digestive gland and branchial heart of cuttlefish (*Sepia officinalis*).¹¹ Thus, cephalopods may serve as vectors of DA and may be victims as well.

Fish

Marine fish are also both vectors and victims of marine biotoxins. Periodic massive fish kills associated with *K. brevis* red tides have been reported since the 1800s in Florida’s coastal waters, where red tides are almost annual events (www.mote.org). For example, in the

1983 event, more than 150 tons of dead fish had to be removed from Tampa Bay. Clinical signs in fish include twisting and corkscrew swimming patterns that are sometimes quite violent, followed by abnormal orientation in the water, rapid shallow respiratory movements, respiratory failure, and death.⁵⁷ Brevetoxin-like compounds may be secreted by HABs from *Heterostigma* spp. flagellates and have been associated with deaths of fish and invertebrates (including starfish) in Washington State.⁴⁴ Postulated mechanisms of lethality include respiratory and cardiac paralysis, excessive mucus production coating the gills, and hypoxia from oxygen consumption by the blooms. No clinical signs other than death were reported, and lesions were not described. Hypoxic and anoxic zones have been reported in connection with blooms of marine algae, including *K. brevis*, such as that which occurred during the 2004–2005 red-tide event on the west coast of Florida near Tampa and Sarasota bays.⁴⁰ These zones develop secondary to the presence of the blooms and decomposition of algal cells, dead fish, and other marine organisms and during the process of eutrophication.²⁹

Fish mortality associated with saxitoxicosis has been reported in chub mackerel (*Scomber japonicus*), herring (*Culpeus harengus*), cod (*Gadus morhua*), and salmon (*Oncorhynchus* spp.).^{7,57,58} Clinical signs other than death have not been described. Other species of fish, such as puffer fish (*Arothron* spp.) and Atlantic mackerel (*Scomber scombrus*), appear to tolerate the toxin, and concentrations of saxitoxin in their livers increase with age and season, reaching a mean toxic concentration of 112.4 mg STX equivalent per 100 g of liver.⁷

Planktivorous fish such as Pacific sardines (*Sardinops sagax*) and northern anchovy (*Engraulis mordax*) are implicated as vectors of DA, and apparently they readily consume toxic diatoms. However, additional studies have confirmed that benthic fish may also contain this biotoxin.^{36,38} Anchovy exposed experimentally to DA have a variety of clinical signs, including spinning, inability to school, disorientation, and death.³⁶ Fish consuming DA or gavaged orally quickly excrete (depurate) the toxin and thus are considered to be dangerous as vectors only during the course of an actual bloom.

Fish embryo and fingerling death and developmental anomalies may be induced experimentally by brevetoxins, saxitoxins, ciguatoxin, and azaspiracid in medaka (*Oryzias latipes*); saxitoxins in milkfish (*Chanos chanos*); and DA in zebra fish (*Danio rerio*).^{8,9,14,30,37,53} Effects have ranged from bradycardia and delayed development (azaspiracid-1) to scoliosis (ciguatoxins, brevetoxins) and encephaloceles (brevetoxin-1).

Sea Turtles

Sea turtle deaths, suspected to be caused by brevetoxinosis, occur in Florida coincident with deaths of manatees, small cetaceans, numerous fish, and benthic organisms (Florida Fish and Wildlife Research Institute). Sea turtle deaths have also been reported associated with red tide resulting from an unspecified dinoflagellate in the waters of Baja California in 1992.^{41,48} As with manatees, green sea turtles may browse on sea grasses, which are shown to retain brevetoxin and dinoflagellates on surface biofilm long after an actual bloom has passed.¹⁷ Sea turtles come ashore either dead or weak and disoriented. Lesions have not yet been described.*

Birds

Seabirds become poisoned by marine biotoxins through the ingestion of prey vectors, including invertebrates and fish. Species of seabirds most often affected include cormorants (*Phalacrocorax* spp.) and brown pelicans (*Pelecanus occidentalis*). In 1993–1994, red-breasted mergansers (*Mergus merganser*) and lesser scaup (*Aythya affinis*) developed weakness, reluctance to fly, slumping of the head, clear nasal discharge, viscous oral discharge, oil gland dysfunction, excessive lacrimation, chalky yellow diarrhea, dyspnea, tachypnea, tachycardia, decreased blood pressure, depressed body temperature, diminished reflexes, and dehydration (Florida Fish and Wildlife Research Institute). During brevetoxin-induced morbidity and mortality in 1997 and 2000, double-crested cormorants (*Phalacrocorax auritus*) from Florida had clinical signs of ataxia with hypermetria, stance with wide leg separation, and head tremors, but no specific histologic lesions were found in the limited number of cormorants examined.³¹ The clinical signs are similar to those observed in *P. auritus* with Newcastle disease (ND), although cormorants with ND have histologic lesions of viral encephalitis.³³ Although *K. brevis* does not occur in California coastal waters, inhaled brevetoxin-like compounds were associated by immunohistochemistry with the deaths of about 400 common murres (*Uria aalge*).²⁷ Gross lesions included serosanguineous rhinitis and pulmonary edema.

Brown pelicans, Brandt's cormorants (*Phalacrocorax penicillatus*), and common loons (*Gavia immer*) develop DA intoxication off the coast of central California and

Baja California.^{18,46,47,59,60} Although many birds come ashore dead, some are found alive with neurologic signs that in pelicans include ataxia, wing droop and extension, pouch scratching and rubbing of the back of the head on the back and wings (possible pruritis), and slow, swinging head motions. Affected cormorants are said to be unusually docile. Lesions in affected pelicans include skeletal and cardiac myonecrosis, and one case of "diffuse cerebral necrosis" has been reported.^{59,60} Diagnosis is made by analysis of gastric contents.

Three species of birds have been inoculated experimentally with DA: domestic pigeons (*Columba livia*), domesticated mallard ducks (*Anas platyrhynchos*), and common murres.⁴⁹ All three species develop abnormal postures and behaviors. Clinical signs in pigeons include agitation with aimless walking and "seeking" movements of the head, wing droop and hock sitting, decreased response to handling, and occasional trancelike static behavior. Signs progress to wing and tail tremors, followed by recovery in most cases. In a few, more severely affected birds, dyspnea and watery nasal discharge occur, followed by seizures and opisthotonos leading to death or euthanasia.

Captive-reared mallard ducks injected intracoelomically initially become quiet and unresponsive to stimuli, after which they develop a horizontal posture with extension of the neck, accompanied by aimless walking, lack of normal flocking behavior, and polyuria. Sternal recumbency, muscle fasciculation, and hyperthermia followed by death occur in a few birds.

Common murres dosed intraperitoneally also show alterations in posture, with wing abduction and abnormal rafting behavior. Higher doses produce hypothermia, weakness, lethargy, abnormal posture in the water, lack of "flight response" when approached, and muscle tremors.

Gross necropsy and histopathology do not reveal any pathognomonic lesions. Pigeons with fatal intoxication develop pulmonary edema and visceral congestion. Cerebellar Purkinje cell necrosis is the only lesion in the nervous system and was seen in only one pigeon. The only lesion in ducks and murres is mild muscle fiber necrosis.

As in other species, DA is rapidly cleared in birds, with an elimination half-life in pigeons of about 2 hours. Domoic acid is cleared from the serum through the kidneys. In experimentally intoxicated birds, peak serum levels are at 30 minutes to 2½ hours, and by 72 hours DA is no longer detectable in serum or urates. Samples of choice for detection of DA in birds are gastric contents, urates, and droppings, followed by kidney tissue and serum. Because of rapid clearance and the

*http://research.myfwc.com/features/view_article.asp?id=2122.

perpetuation of excitatory neurotoxicity by secretion of endogenous glutamates, it may be difficult to correlate levels of DA with clinical signs.

Manatees

Although boat strikes and cold shock continue to be major causes of manatee deaths, there have been mass mortality events caused by brevetoxicosis in 1963, 1982, 1996, 2002, 2003, and 2005 (National Marine Mammal Stranding and Response database).^{5,6,17,35} Exposure is through ingestion (sea grass and tunicates found on sea grass) and inhalation. Sea grass may remain toxic after the bloom is over.¹⁷ Manatees that strand alive have a variety of neurologic signs, including disorientation, incoordination, inability to maintain proper position in the water, hyperflexion, muscle fasciculations, seizures, flaccid paralysis, and dyspnea.^{5,6} Another clinical abnormality reported is consumptive coagulopathy and thrombocytopenia of uncertain pathogenesis. Grossly, nasopharyngeal congestion and pulmonary edema are the most obvious findings after inhalation exposure. All viscera are congested. Histologically, catarrhal and hemorrhagic rhinitis, tracheitis, and bronchiolitis accompany pulmonary congestion, edema, and hemorrhage. Immunohistochemistry has been used to substantiate the diagnosis. Samples yielding the highest concentrations of brevetoxins are stomach contents and liver.

Pinnipeds

Saxitoxicosis is suspected to have been responsible, at least in part, for the mass mortality of Mediterranean monk seals (*Monachus monachus*).¹² Morbilliviruses were also isolated from some of the seals.^{24,42,56}

Along the California coast, California sea lion (*Zalophus californianus*) mortalities associated with blooms of *Pseudonitzschia* spp. have occurred frequently during the past decade. The first well-characterized unusual mortality event (UME) was in 1998⁴⁶ and resulted in the stranding of at least 100 sea lions, mostly females. Clinical signs include stupor, increased docility, ataxia, head weaving, muscle tremors, opisthotonos, titanic seizures, coma, and death.²² Creatine kinase is elevated. Affected sea lions generally would not eat even when seizures were controlled with anticonvulsants. Domoic acid is best detected in urine because vomition appears to occur and stomachs are often empty of ingesta that may be used for analysis.

Myocardial pallor was a striking finding at necropsy of sea lions dying acutely. Gravid females sometimes had twisted or ruptured uteri with intraabdominal delivery.^{22,50} Examination of the brains of animals dying after several days in rehabilitation revealed hippocampal atrophy. Histologic brain lesions are focused on the limbic system and included acute necrosis of the dentate gyrus and of pyramidal cells in sectors CA 4, 3, and 1 of the anterior ventral hippocampus.⁵⁰ In addition, there is a band of vacuolation of the neuropil in the stratum lacunosum-moleculare of the hippocampus. In severe cases, necrosis extends along the anterior ventral hippocampus to include sectors CA 1 and 3 and the laminar cortical neurons of the rhinencephalon, prefrontal cortex, piriform lobe, and amygdala. Animals surviving for longer periods have evidence of hippocampal atrophy with mild lymphocytic perivascular cuffing. Myocardial degeneration, contraction band formation, and necrosis with overlying fibrinous epicarditis correspond to the myocardial pallor appreciated grossly. Interestingly, a syndrome of seizures leading to stranding after DA-producing blooms have passed has been associated with gross and histologic evidence of unilateral hippocampal atrophy.

Suspected mortality from ciguatera fish poisoning in Hawaiian monk seals (*Monachus schauinslandi*) was reported in 1978.^{4,21} Clinical signs were not documented.

Sea Otters

Sea otters in Alaska (*Enhydra lutris kenyoni*) are suspected to have developed saxitoxicosis (PSP), although in experimental studies they avoided contaminated prey items.^{13,34} Clinical signs (other than death) and lesions were not documented. Domoic acid was suspected to be a major component of the Southern sea otter (*E. lutris nereis*) UME in 2003.²⁸ Prey vectors were thought to be mole crabs in some of the cases. CNS lesions are rarely seen, possibly because of the acute nature of the intoxication. When present, the targeted area of the brain appears to be the dentate gyrus and hippocampus, as in the sea lions. Domoic acid has also been linked statistically to dilated cardiomyopathy in sea otters.³²

Cetaceans

Brevetoxicosis has been implicated in bottlenose dolphin mortality events in 1999–2000 and 2004 along the Florida Panhandle, in each of which more than 100 dead dolphins were stranded in a relatively short

period.¹⁷ These events were temporally and spatially associated with red tides and deaths of fish and marine invertebrates.* Dolphin samples positive for PbTx included stomach contents, feces, urine, and liver (in that order). There are no specific gross or histologic lesions reported for brevetoxicosis in dolphins.

Saxitoxicosis has been implicated in the deaths of 14 humpback whales (*Megaptera novaeangliae*) off the coast of New England and eastern Canada in 1987.²⁰ The vector was determined to be Atlantic mackerel (*S. scombrus*).

Domoic acid found in some of the dolphins during in the 2004 Florida UME was not considered to be the principal cause of death. However, DA is the suspected cause of UMEs in common dolphins (*Delphinus* sp.) in 1995–1997 in Mexico^{41,48} and in 2002 and 2003 along the coast of Southern California. Animals that strand alive have seizures. Brain lesions other than edema are not reported. The cetacean hippocampus is much smaller proportionately than that in terrestrial mammals and pinnipeds, and glutamate receptor mapping has not yet been undertaken to identify probable target areas. Domoic acid has also been detected in feces of humpback and blue whales (*Balenoptera musculus*), but mortality was not described.³⁸

DIAGNOSIS OF HARMFUL ALGAL BLOOM INTOXICATION

The diagnosis of HAB intoxication relies on temporal and spatial association with a bloom; detection of the algae in the patient's GI tract; detection of the toxin in GI contents, serum, urine, or other clinical samples; and the presence of compatible lesions in animals succumbing to the putative intoxication. If possible, prey species should be analyzed as well. Immunohistochemical localization of the toxin in tissues has been developed for brevetoxins and is being developed for saxitoxins. The small size of DA (311 daltons, similar to one amino acid) and transient tissue residence time make development of immunohistochemical staining as a diagnostic tool difficult.

If marine biotoxins are suspected, information about testing is available through the toll-free Marine and Fresh Water Toxin Disease Reporting Hotline (1-888-232-8635) operated by the University of Miami NIEHS Marine and Freshwater Biomedical Sciences Center (MFBSC), in cooperation with the Florida

Department of Health (FDH) and the Centers for Disease Control and Prevention (CDC). The hotline operates 24 hours a day, 365 days a year, and may give referrals for laboratory testing. Alternatively, members of the Marine Mammal Health and Stranding Network, National Oceanic and Atmospheric Administration (NOAA), National Marine Fisheries Service (NMFS), and Office of Protected Resources may be able to have samples run through NOAA laboratories. NOAA's marine biotoxin research and testing centers include the Center for Coastal Environmental Health and Biomolecular Research in Charleston, South Carolina,* and the Harmful Algal Blooms Program at the Northwest Fisheries Science Center.[†] The California Animal Health and Food Safety (CAHFS) Laboratory may test for DA.[‡] In Canada, Jellett Rapid Testing (<http://www.jellett.ca>), and in the United Kingdom, Integrin Advanced Biosystems (<http://www.integrin.co.uk>), offer testing for marine biotoxins.

TREATMENT

There are no specific antidotes for the marine biotoxins. Rather, symptomatic treatment and supportive care are used to bide time while the toxin is either eliminated or metabolized by the victim.^{51,52}

Reported treatment for brevetoxicosis in manatees includes steroidal and nonsteroidal antiinflammatory drugs, fluids, nutritional supplementation via gavage, and buoyancy devices to keep the animals floating upright.⁵ Some manatees have recovered and been released.

Experimental intoxication in rats has shown that preventing the seizures will protect neurons against death from DA excitotoxicity. Reported anticonvulsants used in California sea lions with DA intoxication include diazepam at 0.1 to 0.2 mg/kg intramuscularly (IM) up to four times per day, lorazepam at 0.03 to 0.04 mg/kg up to two times daily, and phenobarbitone at 4 mg/kg IM or orally twice daily for 2 days, then 2 mg/kg twice daily.²² Gravid sea lions improve dramatically following spontaneous abortion or induced parturition using a single dose of 500 µg IM of prostaglandin F_{2α}. Subcutaneous lactated Ringer's or 0.9% sodium chloride solution at a rate of 25 mg/kg/day is given until the sea lion begins feeding again.

*http://www.nmfs.noaa.gov/pr/pdfs/health/ume_bottlenose_2004.pdf.

*<http://www.chbr.noaa.gov/default.aspx?category=mb&pageName=biotoxin>.

†<http://www.nwfsc.noaa.gov/hab/>.

‡<http://cahfs.ucdavis.edu/testfees.php>.

Most cases of saxitoxicosis in marine animals have stranded after death. In live strandings, respiratory support, including ventilation, might be necessary.

Mannitol may be helpful in treating ciguateric fish poisoning (CFP) in humans, although results have varied.²⁵ Reports of CFP in marine mammals have been limited to mortalities, so no specific treatment has been suggested.

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CHAPTER 42

Elephant Herpesviruses

LAURA K. RICHMAN

A newly recognized, often-fatal hemorrhagic disease attributed to *elephant endotheliotropic herpesvirus* (EEHV) has been found in North America, Europe, the Middle East, and Asia. At least two closely related herpesviruses are associated with the disease; one is fatal for Asian elephants, and the other may be lethal for African elephants.

The disease attributed to EEHV has a sudden onset and is characterized by subcutaneous edema of the head and proboscis, cyanosis of the tongue, decreased white blood cell and platelet counts, and internal hemorrhages. Histologic abnormalities are predominantly localized to the heart, liver, tongue, and intestinal tract and include the appearance of basophilic intranuclear viral inclusion bodies in the microvasculature of these organs. On electron microscopy the inclusion bodies contain viral capsids morphologically consistent with herpes virions. The cell type affected by this disease demonstrates that this virus has a predilection for endothelial cells (*endotheliotropism*), which is unusual for any of the previously characterized herpesviruses. The high fatality rate is attributed to acute myocardial failure and capillary injury and leakage from endothelial cell damage caused by the presence of the herpesvirus.¹³

Elephant herpesviruses have contributed to a significant proportion of captive-born elephant mortalities. An accurate accounting of this disease has been limited by the unavailability of case material before the 1980s.

ETIOLOGY

Current knowledge and data generated to date suggest that the EEHVs are host specific for elephants. Deoxyribonucleic acid (DNA) extracted from tissues of most of the deceased Asian elephants tested, as well as in the blood of all the survivors treated with famciclovir during their illness, encoded herpesvirus terminase sequences that had only minor variability at the

nucleotide level. Initially, no terminase polymerase chain reaction (PCR) products were obtained from African elephants with endothelial disease. Conversely, DNA polymerase-directed PCR generated products from the African cases only, but not from the Asian elephants with endothelial disease. Once new, specific primers were constructed, PCR products almost identical to each other at the nucleotide level were obtained from the DNA polymerase gene from four Asian elephants. However, sequence comparison of the herpesvirus DNA polymerase regions from the two elephant species showed only a 76% protein identity between the viruses detected in Asian and African elephant cases, with 65% identity at the nucleotide level. This indicates two different species of herpesviruses were present in the elephants.¹³

Similarly, PCR products from the terminase gene region were also obtained from an African elephant once a second set of specific primers were constructed. Sequence comparison of the terminase gene region from the two elephant species showed 80% identity at the nucleotide level, although in this case the changes were all synonymous, and the encoded proteins showed 100% amino acid identity.¹³ Control samples that proved negative included heart and liver tissue from 10 Asian and 5 African elephants that died from conditions unrelated to endothelial disease. Additionally, DNA extracted from peripheral blood of more than 100 asymptomatic Asian and 41 African elephants, including herd mates from facilities where herpesvirus deaths had occurred, were all negative for herpesvirus using PCR primer sets specific for both viruses.

Polymerase chain reaction sequencing of DNA extracted from African elephant cutaneous papillomas and vulval lymphoid patches encoded protein sequences identical to those obtained from the Asian elephants with EEHV disease.¹³ Control samples that proved negative included pustular skin lesions from two African and three Asian elephants, without evidence of inclusion bodies, and suppurative or

noninflammatory vulval lesions from one Asian and two African elephants. On the other hand, DNA extracted from pulmonary tissue of wild African elephants with morphologic evidence of herpesvirus contained viral sequences in the DNA polymerase gene region that had 100% protein identity with the herpesvirus that was fatal for two African elephants.

As of this writing, three skin lesions, six vulval lesions, and one pulmonary lesion from Asian elephants that were grossly and histologically similar to the lesions found in African elephants have not yielded herpesvirus sequences using both direct and redundant primer sets. This may be attributed to the age of the samples (some were over 30 years old and in paraffin blocks). The results obtained to date may indicate that African elephants may harbor two novel herpesviruses: one that causes fatal endotheliotropic disease in Asian elephants and the other that causes the same disease picture in African elephants.

The proteins encoded by the PCR-amplified DNA obtained from each of the Asian and African elephants that were affected with the disease are clearly those of herpesviruses, but they are distinct from any of the currently known herpesviruses. By comparing amino acid sequences that are characteristic for each herpesvirus subfamily, together with the results of a phylogenetic tree analysis, the terminase protein of the elephant herpesviruses shows slightly greater similarity to betaherpesviruses than to alphaherpesviruses or gammaherpesviruses, but it is clearly not that of a cytomegalovirus (CMV). Similarly, the elephant virus DNA polymerase proteins do not fit into any of the herpesvirus subgroups.¹³ These findings within these two highly conserved herpesvirus gene regions, together with the unique pathogenesis, suggest that the causative agents of elephant endothelial disease are either outliers of mammalian betaherpesviruses or belong in a previously unrecognized subfamily.

After extensive sequencing of lambda libraries constructed from diseased tissue from two Asian and one African elephant, it is clear that the EEHVs are unique. Although most of the conserved genes align with the betaherpesviruses, and EEHVs probably contain some beta-specific genes, namely UL82/83 and UL88 (using the human CMV nomenclature), a thymidine kinase (TK) gene homolog is present in both African and Asian EEHV genomes that is not present in any betaherpesvirus sequenced.¹¹ The compelling part of this finding is not only the presence of the TK gene, but that it is adjacent to genes aligning with betaherpesviruses. Furthermore, the African EEHV genome contains both the large and the small subunits of

ribonucleotide reductase (RR).¹¹ No other betaherpesvirus contains both subunits of RR, but alphaherpesviruses and gammaherpesviruses contain both RR1 and RR2.

Based on these sequence data, the International Committee of Taxonomy of Viruses is considering classification of the EEHVs as a new subgroup within the betaherpesviruses.

EPIDEMIOLOGY

All herpesviruses may persist in their host in the form of an episome within the nucleus of various cell types. Probably every vertebrate species has at least one herpesvirus that has evolved with the host for millions of years; humans have at least nine herpesviruses. Generally, the natural host range of a herpesvirus is restricted to one species, although there are exceptions. Transmission from one host species to another may occur; for example, simian herpesvirus B ("B virus"), although innocuous in macaques, may be transmitted to humans and is almost invariably fatal.^{17,18} Herpesviruses are usually highly adapted to their host and rarely cause a lethal disease, except in very young or immunocompromised individuals. Latent herpesvirus infection may be a reservoir of virus that is shed either frequently in excretions or intermittently in lesions.

Before 1995, there were sporadic reports of herpesviruses in elephants. Proliferative cutaneous lesions were described in a herd of captive African elephants.⁷ The lesions from several of these elephants were removed and examined histologically and ultrastructurally. There was morphologic evidence of herpesvirus within the epidermal cells of the lesions. Consensus primer PCR combined with sequencing yielded molecular evidence that confirmed the presence of herpesvirus sequences identical to those found in Asian elephants with disseminated EEHV disease.¹³ This finding suggests that at least some of the Asian elephant deaths were potentially caused by cross-species infection with a herpesvirus that is naturally latent in, but normally not lethal to, African elephants.

Vesicles and plaques in the distal urogenital tract of both Asian and African elephants have also been described.^{1,10} The lesions have been seen in 62% of captive Asian elephants, 89% of captive African elephants, and 90% of free-ranging African elephants. Histologically, they are composed of reactive lymphoid follicles. Only rarely are intranuclear inclusions seen within the lesions; they are present within dendritic cells scattered in the lymphoid tissue.¹ Con-

sensus primer PCR for two of five lesions tested in African elephants again yielded molecular evidence of a herpesvirus identical to the EEHV that causes lethal disease in Asian elephants.¹³ Similar lesions in Asian elephants have not yielded any herpesvirus sequences by PCR.

The third herpesvirus-associated lesions found in African elephants are lung nodules. The nodules are usually gray and vary from spongy to firm in consistency. They are small, up to 3 cm in cross-sectional diameter, and multiple nodules may be found within the same elephant. The nodules have been seen in about 80% of free-ranging African elephants at Kruger National Park.⁸ Fourteen lung nodules from 10 African elephants were obtained, and PCR sequencing yielded molecular evidence of the same strain of EEHV that is lethal for African elephants.¹² Therefore, African elephants carry at least two herpesviruses: one that may be lethal to Asian elephants and the other that is fatal in young African elephants. The status of the Asian elephant as a carrier for either virus has yet to be determined; in closed Asian herds, however, deaths have been attributed to EEHV with no history of exposure to African elephants.

Two genera of elephants are alive today (*Loxodonta* and *Elephas*) and originated from a common ancestor, along with *Mammuthus*, about 3 million years ago. *Loxodonta* has recently been separated into two distinct species: *L. africana* (savannah elephant) and *L. cyclotis* (forest elephant), based on DNA sequence variation in four nuclear genes.¹⁴ All modern elephant species migrated out of Africa, and the two African species are separated by geographic barriers, but rare intermingling and breeding do occur. In the last few hundred years, 3 million years of species separation has been challenged by the advent of zoos and wildlife parks that sometimes house both species in the same enclosure. The data presented may indicate the existence of a herpesvirus that is indigenous to and nonlethal in African elephants (perhaps latent in the lymphoid patches and productive in the papillomas). When this virus is present in captive African elephants and inappropriately cross-infects young Asian elephants, the resulting primary disease is apparently lethal. The status of herpesviruses in wild Asian elephants is currently unknown, but a limited survey of lymph node biopsies of Asian elephants in Thailand failed to yield herpesvirus sequences by PCR using direct primers for EEHV.⁵

Several closed Asian elephant herds have experienced one or more cases of disseminated EEHV, and there is no history of either direct or indirect exposure

to African elephants. Additionally, an Asian elephant in Europe delivered a term stillborn fetus with histologic and molecular evidence of EEHV in the placenta and tissues of the fetus. The dam remained completely healthy during this time. This event represents either primary infection of the dam during her gestation period or reactivation of endogenous EEHV. There have been several cases of stillborn Asian elephant fetuses with EEHV in target organs, so it is likely that the virus is at least contributory to the high stillbirth rate in captivity.² It is still plausible that the EEHV lethal for Asian elephants originated from the African species and has been transmitted to and circulates within the Asian elephant population in captive settings. Some elephants likely develop asymptomatic infections or simply a mild illness that goes unnoticed, then intermittently shed the virus. Young elephants clearly are more susceptible to severe, disseminated illness because a majority of elephant deaths have occurred between 1 and 2.5 years of age. This pattern of transmission has similarities to human herpesviral disease.

Several adult Asian elephants with EEHV have died, and most had similar histories of being housed in isolation for most of their lives. Once the elephants were moved to a new zoo or another elephant entered their facility, they died of EEHV disease. Primary exposure likely occurred when the elephants were moved, and because the animals were immunologically naive, severe illness and death ensued. The correct conditions for primary exposure to EEHV, if the elephant is to survive or be asymptomatic, are not entirely clear, but with the human betaherpesvirus HHV-6, there is individual variation in clinical signs on primary exposure. Some infants develop only a mild rash, whereas others have severe illnesses, including encephalitis and seizures.^{3,6,9} Strain and host variation may affect disease outcome. A similar situation might also apply to the disease associated with the elephant herpesviruses.

CLINICAL SIGNS

Clinical evaluations of the recent cases of EEHV disease revealed that the most common clinical signs were lethargy, anorexia, mild colic, and edema of the head, proboscis, neck, and thoracic limbs. In several elephants, lingual cyanosis, oral ulcers, and diarrhea have been noted. In a few of the elephants successfully treated with famciclovir (Novartis), lingual cyanosis and head and neck edema resolved over 1 week.¹⁵

DIAGNOSIS

Clinical Pathology

Asian elephants with disseminated EEHV may become lymphopenic, thrombocytopenic, anemic, and dehydrated.^{13,15} In the index case of EEHV in an Asian elephant, aerobic and anaerobic bacterial cultures of heart blood, pericardial and peritoneal fluid, cerebrospinal fluid, axillary lymph node, and liver were negative, and bacterial cultures of multiple segments of the gastrointestinal tract yielded no enteric pathogens. Cocultivation of the liver and heart were negative for any of the known herpesviruses or any other virus known to infect elephants.

Antemortem Diagnosis

Antemortem diagnosis of disseminated EEHV may be performed by PCR on a sample of whole blood, using specific primers directed toward the elephant herpesviruses.¹³ All elephants that have the classical clinical signs and lesions associated with EEHV have been positive for the virus on PCR.

Differential Diagnosis

Where appropriate, encephalomyocarditis virus (EMCV) should be ruled out. Other diseases to consider include leptospirosis, other bacterial septicemias, toxicities, and nutritional deficiencies. There are reports of blister beetles causing oral ulcers in elephants.⁴

Postmortem Findings

Gross findings of elephants that have died with EEHV include pericardial effusion with extensive petechial to ecchymotic hemorrhages involving the epicardial and endocardial heart surfaces and throughout the myocardium. Diffusely scattered petechiae within all the visceral and parietal peritoneal serous membranes are also seen, as well as cyanosis and petechial hemorrhages on the tongue, hepatomegaly, and more variably, oral, laryngeal, and large intestinal ulcers. The microscopic findings consist of extensive microhemorrhages throughout the heart and tongue associated with edema, and mild infiltrates of lymphocytes, monocytes, and neutrophils between myofibers. Multifocal changes include hepatic sinusoidal expansion with mild subacute inflammation and mild hepatocellular

vacuolar degeneration. The capillary endothelial cells in the myocardium, tongue muscle, and within the hepatic sinusoids of the liver contain amphophilic to basophilic intranuclear viral inclusion bodies that are closely associated with the microhemorrhages (Figures 41-1 and 42-2). Ultrastructural studies of the endothelial inclusion bodies reveal 80- to 92-nm-diameter nucleocapsids morphologically consistent with the herpesvirus group.^{12,13} The herpesvirus particles are most often present within the nucleus and rarely the cytoplasm, but have not been seen intercellularly.

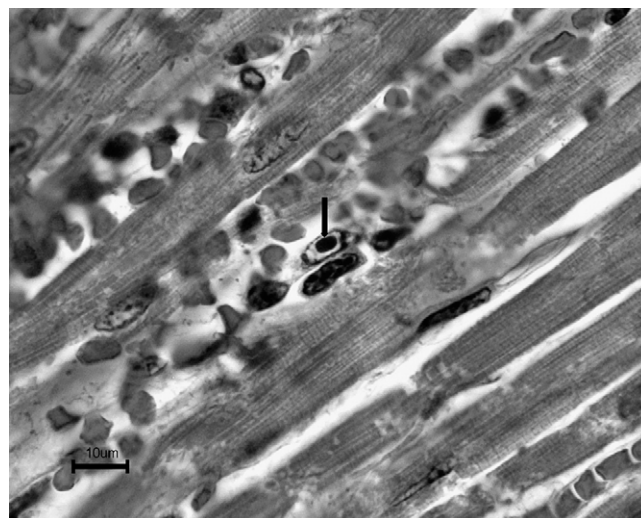


Fig 42-1 Photomicrograph of Asian elephant heart showing inclusion body (arrow) characteristic of elephant endotheliotropic herpesvirus (EEHV). (See Color Plate 42-1.)

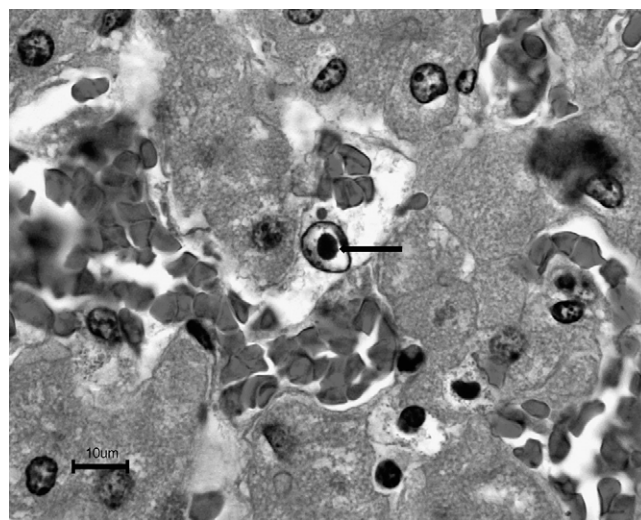


Fig 42-2 Photomicrograph of Asian elephant liver showing inclusion body (arrow) characteristic of EEHV. (See Color Plate 42-2.)

THERAPY

Treatment with the human antiviral drug famciclovir has been attempted in several Asian elephants with confirmed disseminated EEHV infection; less than half of those treated survived.¹⁵ Famciclovir is effective against human herpesviruses 1 and 2 and varicella-zoster virus (VZV) by selective inhibition of herpesviral DNA synthesis. Its efficacy has not been determined for herpesviruses other than those that infect humans. The first elephant treated with famciclovir survived, and plasma levels of penciclovir were assessed throughout the treatment period.^{12,15} Possible explanations for treatment failure in subsequent cases include inadequate dosage, delayed treatment onset, viral factors such as TK variation or mutation, and undetermined host factors. Additional antiviral drug therapies have not yet been attempted.

PREVENTION

Serologic determination of previous exposure to herpesvirus infections in other animals has been accomplished by screening for antibodies to one or more of the herpesvirus antigenic proteins. Glycoprotein B (gB) is the most common herpesvirus protein used for serologic detection and is useful because it is highly antigenic. Glycoprotein B is also a key protein for determination of strain variability between herpesviruses; for example, HHV-6 A and B may be discerned serologically based on gB epitope diversity.¹⁶

A nationwide serosurvey for each of the strains of EEHV is underway using an indirect enzyme-linked immunosorbent assay (ELISA) test directed to an antigenic portion of EEHV gB.¹¹ In one Asian elephant, serum samples obtained 10 months before and 2 months after EEHV illness showed a 12-fold increase in titer. Using banked serum samples, both retrospective and prospective epidemiologic studies are underway, and results will be correlated to documented illnesses and deaths within an elephant herd.

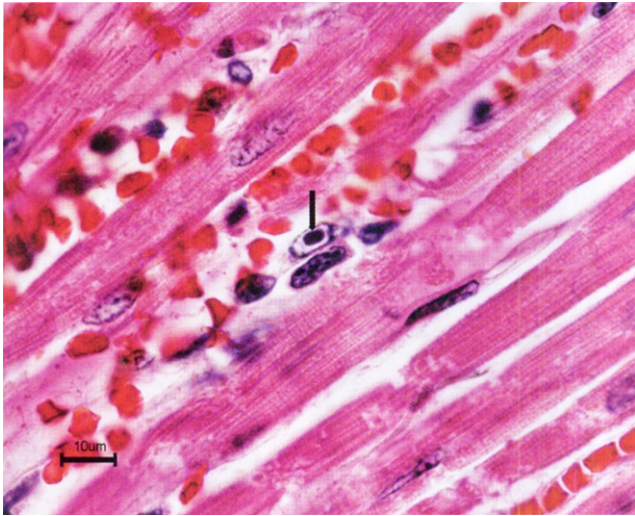
Once titer trends within a herd have been analyzed, patterns might emerge that will aid in herd management and elephant movement decisions mediated by regional elephant management plans, such as the Association of Zoos and Aquariums' (AZA) Species Survival Plan (SSP). Currently, reproductive capability to produce a genetically stable population in captive North American elephants is in jeopardy and may require increased elephant movement for breeding purposes. A validated ELISA test to detect EEHV-exposed elephants or potential carriers will be impor-

tant to reduce at-risk reproductive activities and the production of more young, susceptible elephants.

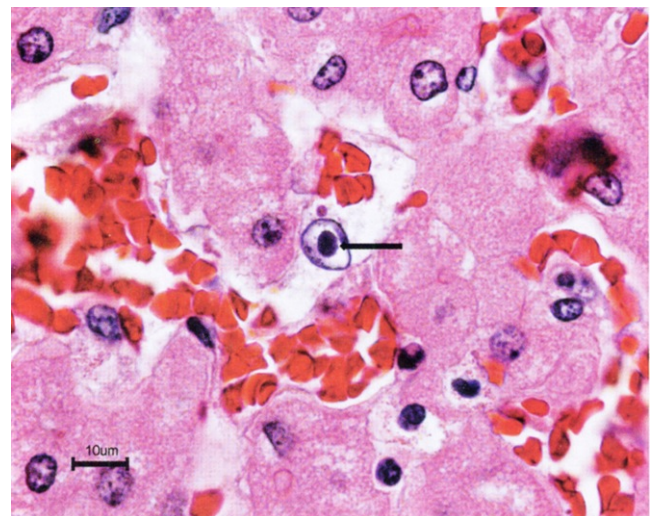
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Color Plate 42-1 Photomicrograph of Asian elephant heart showing inclusion body (*arrow*) characteristic of elephant endotheliotropic herpesvirus (EEHV). (For text mention, see Chapter 42, p. 352.)



Color Plate 42-2 Photomicrograph of Asian elephant liver showing inclusion body (*arrow*) characteristic of EEHV. (For text mention, see Chapter 42, p. 352.)

Tuberculosis in Elephants

SUSAN K. MIKOTA

Tuberculosis (TB) is an ancient disease of both humans and animals. Tuberculous scarring has been observed on bones from 21 of 48 mastodon skeletons recovered in North America (dating to the last Ice Age), and deoxyribonucleic acid (DNA) from the human form of TB has been isolated from a 17,000-year-old bison bone.²⁴ Tuberculosis and its treatment in elephants was described by Ayurvedic physicians in Asia more than 2000 years ago.^{28,40}

A case of TB in an elephant at the London Zoo in 1875 was the first published report in modern times,¹⁷ although the archives of the European Elephant Group indicate an even earlier case in an Asian bull named “Hans” who died in 1802 from TB at Jardin des Plantes, Paris, at age 18 years.¹⁶ Numerous case reports appeared in the literature in the twentieth century.*

I examined medical records of 379 elephants from 1908 to 1992 and found only eight cases of TB.⁴² This retrospective study’s failure to reveal the significance of TB for elephants may be attributed to the sample population, which included elephants from Elephant Species Survival Program (SSP)–participating zoos in North America, but not privately owned elephants (from which records were not available). In addition, surveillance for TB among elephants even in zoos lacked uniformity and consisted largely of intradermal testing, now known to be inaccurate. Also, routine necropsies of elephants and evaluation of ill elephants for the presence of TB were not performed.

The year 1996 is often heralded as the date that TB “emerged” as a disease of concern for elephants, with outbreaks of TB in elephants in North America.^{4,43,44} Subsequent outbreaks in Europe have since been reported.^{18,34,45}

This chapter references TB in humans because this most closely provides a model for the disease as it is observed in elephants. Significant progress has been achieved since 1996 in understanding TB in elephants,

and although these elephants have benefitted from the knowledge of how TB acts in humans, certain aspects of diagnosis and treatment unique to elephants have evolved.

DEFINITION

Tuberculosis is caused by bacteria in the genus *Mycobacterium*, which comprises more than 100 species. Mycobacteria infect a broad range of species, including humans, nonhuman primates, domestic and nondomestic ungulates and carnivores, marine mammals, psittacine birds, reptiles, and fish. Species susceptibility to specific mycobacteria varies.⁴⁶

In mammals the term *tuberculosis* defines disease caused by *Mycobacterium tuberculosis*–complex organisms. The *M. tuberculosis* complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae*, and *M. pinnipedii*. A vaccine strain derived from *M. bovis* (*M. bovis* BCG) is sometimes listed as a separate member of this complex.

The term *mycobacteriosis* describes disease caused by nontuberculous mycobacteria (NTM), also called “atypical mycobacteria” or “mycobacteria other than TB” (MOTT). Most NTM are saprophytes found in soil or water, but they may occasionally cause disease in humans and animals. *Mycobacterium elephantis*, a rapidly growing, newly described mycobacterium, was isolated from a lung abscess of an elephant that died of chronic respiratory disease.⁶¹ This same organism was isolated from 10 human sputum samples and one human lymph node specimen in Canada; however, there was no epidemiologic link between these reports.⁶³

ETIOLOGY

Mycobacterium tuberculosis (*M. tb*) is the predominant disease-causing agent in elephants, although TB cases

*References 3, 6, 12, 19–22, 47, 53, 58, 60, 62.

have been caused by *M. bovis*. *Mycobacterium szulgai*, an uncommon NTM species, has been associated with fatal disease in two African elephants.³⁰ *Mycobacterium avium* is often isolated from elephants⁵⁰ but has not been associated with clinical disease.

EPIDEMIOLOGY

Frequency and Distribution

Both African and Asian elephants are susceptible to TB. I am aware of 34 known cases of TB affecting 31 Asian and 3 African elephants in the United States between 1994 and 2005.¹⁵ Five cases were reported in Europe in 2002,³⁴ with two subsequent cases in 2005.⁴⁵ More frequent reporting of the disease in Asian but not African elephants may reflect closer human contact related to the use of Asian elephants for performances and rides. Likewise, the vast majority of cases occurring in female elephants may be a result of their higher number in captivity and the greater likelihood of human contact with cows than bulls. The disease occurs in captive elephants in Asia as well.^{6-10,47,54,55} There are no reports to date of TB in wild elephants.

Reservoirs

The reservoirs for *M. tb* and *M. bovis* are infected humans and cattle, respectively.²⁵ Transmission may occur via respiratory or alimentary routes. Feces, urine, genital discharges, milk, and feed or water may contain contaminated droplets. In elephants, *M. tb* has been isolated from respiratory secretions, trunk washes, feces, and vaginal discharges. The *M. avium-intracellulare* group can survive in the environment and, unlike the *M. tb*-complex group, does not need a host to survive. Thus, infection is not always acquired from another infected animal. Clinical disease in elephants from *M. avium* has not been reported.

PATHOGENESIS

Infection versus Disease

Infection with mycobacterial organisms does not necessarily imply active disease. Several possible scenarios may result after exposure to an infectious source (Table 43-1). Tuberculosis is estimated to infect latently one third of the global human population and causes approximately 3 million deaths yearly. Latent TB infection (LTBI) in humans is characterized by a positive intradermal test but a lack of clinical disease and no evidence of active shedding of live bacilli. In LTBI, bacterial organisms are sequestered but may reactivate at a later date. Individuals with LTBI are a reservoir for future active cases, and although only an estimated 4% to 10% of latently infected humans with normal immune status will develop active TB during their lifetime, the identification and treatment of individuals with LTBI and at high risk for activation remain an effective means of control.⁴⁸

Host Immunity

The immune response to TB infection is both cell mediated and humoral. A comprehensive discussion is beyond the scope of this chapter, but Figure 43-1 presents a basic outline. For further details, see Schulger and Rom,⁵⁹ Salgame,⁵⁷ and Boomershtine and Zwilling⁵ or current immunology texts.

CLINICAL SIGNS

Tuberculosis is characteristically a chronic wasting disease; thus the term “consumption” was used to describe the disease in humans in the early twentieth century. Signs in elephants may include weight loss, wasting, and weakness.^{22,40,44,58} Coughing or dyspnea have been reported^{15,56,60} but appear to be uncommon.

Table 43-1

Possible Scenarios after Exposure to Active Tuberculosis (TB)

Scenario	Pathogenesis	Tuberculin Skin Test Status (In Humans)
1	All bacteria are killed, and no disease results.	Negative
2	Bacteria multiply, and clinical disease results (primary TB).	Positive
3	Bacteria become dormant and never cause disease (latent TB).	Positive
4	Latent organisms reactivate and cause active disease.	Positive

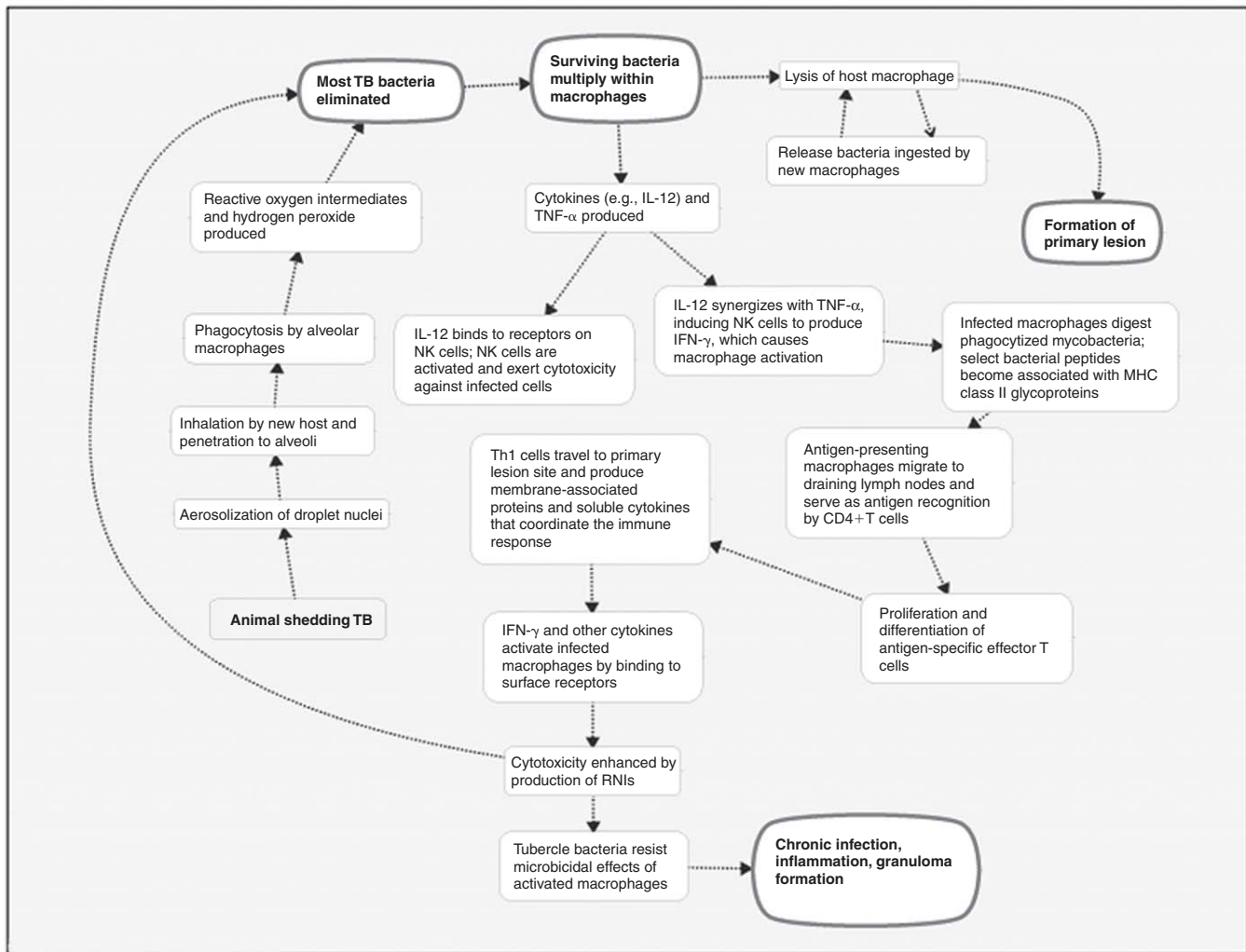


Fig 43-1 Basic outline of immune response after tuberculosis (TB) exposure. Although not illustrated in the chart, note that TB is able to downregulate interleukin-12 (IL-12) and tissue necrosis factor alpha (TNF α) production. NK, Natural killer cells; IFN γ , interferon gamma; MHC, major histocompatibility complex; RNIs, reactive nitrogen intermediates; Th1, T helper type 1.

Exercise intolerance may be noted in working elephants. Ventral edema has been reported, but other pathologic factors may have been the inciting cause.^{53,60} In many cases, clinical signs are absent. Of the 34 cases mentioned earlier, 12 were asymptomatic and diagnosed postmortem. In some of these cases, TB was considered an incidental finding. Elephants that show wasting antemortem will have advanced and possibly disseminated disease on postmortem examination.

DIAGNOSIS

Antemortem Tests

The reader should consult the most current online version of the U.S. Department of Agriculture's *Guidelines for the Control of Tuberculosis in Elephants*.⁶⁴

Direct Tests

Direct tests detect mycobacterial organisms. Direct tests include acid-fast staining, culture, and nucleic acid amplification procedures such as polymerase chain reaction (PCR).

Acid-Fast Bacteria Stain. Mycobacteria do not readily accept Gram's stain but may be detected by acid-fast bacteria (AFB) stains such as Ziehl-Neelsen. A positive AFB trunk wash smear is suggestive of TB but not definitive because other organisms (e.g., *Nocardia*) are also acid-fast. In general, acid-fast staining has low sensitivity (50% in humans) and is nonspecific, particularly in geographic areas where NTM are typically isolated.¹¹

Culture. Isolation of mycobacterial organisms is the method identified in the 2003 *Guidelines*⁶⁴ to establish

a definitive diagnosis of TB in elephants. A trunk wash technique is used in which (1) approximately 60 mL of sterile saline is instilled into the trunk, (2) the trunk is elevated for 20 to 30 seconds, (3) the trunk is lowered into a zippered plastic bag, (4) the elephant is told to forcibly exhale (the usual command is “blow”), and (5) the sample is transferred to a secure screw-top tube for submission to the laboratory. Samples should be sent only to laboratories capable of culturing mycobacteria. Three samples collected on separate days within 7 days are required. The *Guidelines*⁶⁴ include a detailed description of the procedure, or see Isaza and Ketz.²⁶ Elephants must be trained to accept the procedure.

Mycobacteria are slow growing, and results may take 8 weeks or longer. Some laboratories report results as “*M. tb* complex,” and speciation must be requested to distinguish between the closely related *M. tb* and *M. bovis* (speciation is available at the National Veterinary Services Laboratories in Ames, Iowa). Culture is unlikely to yield a false-positive result, but sensitivity is low, and infected elephants may fail to be identified. The primary reasons for trunk wash having a low yield are that TB organisms are shed intermittently (the reason for collecting three samples), and the trunk wash technique may not produce a sample from the lower respiratory tract unless the elephant is well trained to exhale forcibly. Contamination and bacterial overgrowth of samples may occur. In cases in which overgrowth is a problem, cleaning the tip of the trunk before the procedure may help. Cultures reported as “overgrown” should be repeated.

Despite limitations, culture is an important technique, and isolation of the organism is necessary to determine drug sensitivities in TB-positive elephants. The acid-fast smear may detect approximately 1000 colony-forming units (CFUs) per mL of concentrated specimen. Increased yields are obtained by immunofluorescent staining and detection of acid-fast bacilli. By comparison, culture should be able to detect 100 CFUs/mL.

Nucleic Acid Amplification Techniques. The Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test (MTD; Gen-Probe, San Diego, CA 92121 USA) utilizes transcription-mediated amplification (TMA) and the hybridization protection assay (HPA) to qualitatively detect *M. tb*-complex ribosomal ribonucleic acid (rRNA). The MTD test will detect rRNA from both cultivable and noncultivable organisms. The MTD is a U.S. Food and Drug Administration (FDA)-approved test for the diagnosis of TB in humans and must be performed in conjunction with culture. This test is only

approved to be used with sediments prepared from sputum, tracheal aspirates, or bronchial specimens. A negative test does not exclude the possibility of isolating an *M. tb*-complex organism from the specimen.

The MTD has a rapid turnaround time (2.5-3.5 hours) and can detect low numbers of organisms. In smear-positive patients, the MTD is comparable to culture with BACTEC 460, and both tests demonstrate a sensitivity and specificity of about 96%. In smear-negative patients, the sensitivity is 72% and the specificity 99%. In one study the MTD was positive on 14 elephants from which *M. tb* or *M. bovis* was cultured, positive on 15 elephants from which TB was not isolated, and negative on 6 culture-positive elephants.⁵⁰ A positive MTD and negative culture result may represent infection with low-level shedding below the detection capabilities of culture or nonviable organisms. Failure of the MTD to detect the 6 culture-positive elephants may be explained by improper specimen collection and transport, specimen sampling variability, laboratory procedural errors, inhibitors, sample misidentification, or transcriptional errors. (The test is not validated for elephants and never will be.) Further research is needed.

A PCR technique is under investigation to detect mycobacterial organisms in trunk wash samples. Experimentally, this PCR has detected very small numbers of mycobacteria using *M. bovis*-spiked trunk washes, but further work is needed to better determine test sensitivity and specificity.³²

Indirect Tests

Indirect tests detect antigen or antibody or measure cellular reactivity against mycobacterial antigen. Indirect tests include the intradermal tuberculin test and serologic assays such as enzyme-linked immunosorbent assay (ELISA).

Intradermal Tuberculin Test. The intradermal tuberculin test detects cellular reactivity against mycobacterial antigens. Although widely used as a screening test in humans and cattle, the intradermal test correlates poorly with culture in elephants^{34,44} and in one study demonstrated a sensitivity of only 16.7%.⁴⁴ This test is not recommended for elephants.

Enzyme-Linked Immunosorbent Assay. The ELISA measures antibodies against specific antigens. A study using a six-antigen ELISA demonstrated an estimated sensitivity of 100% and specificity of 100%, on a limited sample size of 47 Asian and African elephants (7 culture positive).³³ A modified version of

this ELISA, with increased numbers of positive and negative Asian elephants, has thus far had a similar specificity and sensitivity.³² The ELISA remains under investigation and is not commercially available.

ElephantTB StatPak and Multiantigen Print Immunoassay. A rapid serologic test (ElephantTB StatPak, ChemBio Diagnostic Systems, Medford, NY 11763 USA) has been under investigation for the diagnosis of TB in elephants and other host species and may be available soon commercially. The ElephantTB StatPak is an antibody detection immunoassay based on lateral-flow technology. The ElephantTB StatPak incorporates a unique cocktail of recombinant mycobacterial antigens impregnated on a nitrocellulose membrane and placed in a plastic cassette similar to a pregnancy test kit (Figure 43-2). Serum, plasma, or whole blood may be used, and results are available in 20 minutes.

The multiantigen print immunoassay (MAPIA) is a laboratory procedure for antibody detection that uses a panel of multiple recombinant antigens of *M. tb* and *M. bovis* that are applied separately to a nitrocellulose membrane using an automated printing device.³⁶ Elephant serum or a whole-blood sample is incubated with a MAPIA strip, and antigen-bound antibodies are visualized using a species-specific immunoglobulin G (IgG)-binding enzyme conjugate and corresponding substrate.

To date, 99 Asian and 72 African elephants in Europe, Australia, South Africa, and the United States have been tested using the ElephantTB StatPak and MAPIA. These included 21 elephants with TB confirmed by culture. Preliminary data have demonstrated a 100% sensitivity and 97% specificity for the

ElephantTB StatPak and a 100% sensitivity and 100% specificity for the MAPIA using culture as the reference standard³⁵ (Table 43-2).

If the ElephantTB StatPak is used as a screening test and the MAPIA is sequentially applied as a confirmatory assay, the accuracy of this testing algorithm is 100%. Seroconversion on the ElephantTB StatPak and MAPIA has been noted in several elephants months to years before a positive culture. In one elephant that was euthanized and determined to be TB positive, retrospective evaluation of serum indicated seroconversion 8 years earlier. Another important advantage of serodiagnosis of TB in elephants over trunk wash culture is that, once established, the antibody response remains sustained throughout infection and disease, whereas culture may be intermittently positive or negative in infected elephants. A decline in specific antibodies to certain antigens in MAPIA has been observed in five culture-positive elephants that have undergone treatment, suggesting that this technology may also be a useful for monitoring response to therapy.

Gamma Interferon. A gamma-interferon (IFN- γ) assay for elephants that measures the induction of IFN- γ by peripheral mononuclear cells is under development but is unlikely to be available for several years. Two tests have been commercially developed for use in humans; one (QuantiFERON-TB Gold) is FDA approved and available in the United States, and the second test kit (TB SPOT) is approved for use in Europe.

Immunoblot Assay. Immunoblot detects antibodies to bacterial antigens that appear as discrete bands on a nitrocellulose membrane following electrophoresis. Experimentally, immunoblot detected antibody responses to an *M. bovis* whole-cell sonicate in TB-infected elephants 4 years before culture of *M. tb* from trunk washes.⁶⁵

Other Tests

Clinical Pathology. Although clinical pathology cannot provide a definitive diagnosis of TB, a comparative study has shown statistically significant differences in certain hematology and serum chemistry values in TB-infected versus noninfected elephants. Values for A/G ratio, mean hemoglobin concentration, and glucose were lower, and platelets, band neutrophils, eosinophils, calcium, and bicarbonate were higher in culture-positive elephants that were shedding at the time of sampling compared with 20 clinically healthy, culture-negative elephants.²³



Fig 43-2 ElephantTB StatPak (ChemBio Diagnostic Systems). Left, Nonreactive test; right, reactive test.

Table 43-2

Serodiagnosis of Tuberculosis (TB; *M. tb* and *M. bovis*) in Elephants Using the ElephantTB StatPak and MAPIA

Culture status	Number of Elephants	StatPak Positive	MAPIA Positive
Culture negative, nonexposed, healthy or other than TB diseases	115	4*	0
Culture positive for TB	21	21	21
Exposed to known case(s) of TB, trunk wash culture negative, asymptomatic	35	18	13

Data from K. Lyashchenko, Chembio Diagnostic Systems.

*The false-positive results include two cases of *M. szulgai* that were not confirmed by multiantigen print immunoassay (MAPIA pattern differed significantly from that observed for *M. tb*) and one case of an elephant with joint disease and osteomyelitis that may not have been adequately investigated at postmortem to rule out TB.

Restriction Fragment Length Polymorphism (RFLP). Also known as “DNA fingerprinting,” RFLP is used to identify and differentiate mycobacterial strains. In a study of 12 culture-positive elephants from six U.S. herds, six distinct *M. tb* strains were identified, of which three appeared closely related.⁴⁴ In the European outbreak, five elephants and one giraffe were infected by four different *M. tb* strains.³⁴

Spoligotyping. Spoligotyping is a form of PCR for further determining the relatedness between strains. It is based on the DR (direct repeat) locus in the genome of *M. tb* complex.³⁴ The procedure can also be used on formalin-fixed tissues to distinguish *M. tb* from other mycobacteria.⁴⁶

Postmortem Findings

Elephants that die or are euthanized should undergo a thorough postmortem examination according to the procedures outlined in the SSP Elephant Necropsy Protocol.¹⁴ This document delineates specific precautions to protect personnel from infection if TB is known or suspected.

Pathologic changes in elephants infected with TB are found primarily in the lungs and thoracic lymph nodes, although extrapulmonary and disseminated TB may occur involving the liver, kidney, spleen, adrenals, and genitourinary tract. Pulmonary lesions may be focal or widespread, depending on the stage of the disease. Firm granulomatous nodules may be seen in the lungs and bronchial lymph nodes in less progressed cases, and caseous foci may be present. In advanced cases, extensive caseocalcareous and cavitating lesions may be seen throughout the lung parenchyma, often associated with large pulmonary

abscesses colonized by secondary bacteria.⁴³ Enlargement of bronchial and thoracic lymph nodes is common. In one elephant with granulomatous osteomyelitis and necrosis of the femoral head, *M. szulgai* was isolated from the lungs and coxofemoral joint.³¹

Histopathology of early-stage lesions is characterized by epithelioid granulomas with giant cell formation, whereas later-stage lesions are more caseous, with extensive pyogranulomatous pneumonia. Acid-fast bacteria may be sparse.⁴³

Differential Diagnosis

Dental disorders may affect appetite and lead to weight loss. Pulmonary infections caused by other organisms, osteoarthritis, and degenerative diseases affecting the major body organs may present with similar signs as TB.

MANAGEMENT

The reader should consult the online *Guidelines*⁶⁴ for the most current recommendations.

The treatment regimen for elephants with TB is based on human protocols,¹ although the period of treatment for elephants is 12 months versus 6 months for humans. The basic approach is to administer three drugs for 2 months followed by two drugs for 10 months. Isoniazid (INH), pyrazinamide (PZA), rifampin (RIF), ethambutol (ETH), and streptomycin are considered first-line drugs. Note that pyrazinamide is not effective against *M. bovis*. In the case of multidrug-resistant TB (defined as resistance to both INH and RIF), second-line drugs such as amikacin, ciprofloxacin, and levofloxacin may be needed. Consult Peloquin⁵²

Table 43-3

First-Line Antituberculosis Drugs in Elephants and Side Effects

Drug	Route	Starting Dosage (mg/kg)	Target Serum Level at 2 Hours (µg/mL)	Reported Side Effects in Elephants ^{44,64}	Comments
Isoniazid	Oral or rectal	5*	3-5	Anorexia, lethargy, pica, anemia, elevated liver enzymes	—
Pyrazinamide	Oral or rectal	30	20-60	None reported; hepatotoxicity possible	Not effective against <i>M. bovis</i>
Rifampin	Oral only	10	8-24	None reported; hepatotoxicity seen in humans	Adequate levels have not been achieved rectally.
Ethambutol	Oral	30	2-6	None reported; optic neuritis and decreased visual acuity seen in humans	Irritating rectally and rapidly expelled

*Note that 5 mg/kg is the starting dosage suggested in the *Guidelines*.⁶⁴ Based on pharmacokinetic data, 4 mg/kg is recommended when using isoniazid (INH) powder mixed just before administration and 7.5 mg/kg when INH is purchased as a premixed concentrate.³⁹

for a detailed description of the clinical pharmacology of anti-TB drugs. Anti-TB drugs are expensive, and the cost to treat one elephant for a year may exceed \$50,000, excluding laboratory costs to monitor drug levels. Table 43-3 lists recommended starting dosages.

Anti-TB drugs may be given by direct oral or rectal administration. Adequate and reliable drug levels cannot be achieved if drugs are mixed with food and administered “free choice.” For oral administration, some elephants can be trained to accept a bite block, and medications can be delivered through a large animal dose syringe. Most elephants can be readily trained to accept rectal administration, and adequate blood levels can be achieved. For a further discussion of medication techniques for elephants, see Isaza and Hunter.²⁷

Therapeutic drug monitoring is essential for elephants receiving anti-TB drugs.⁵¹ Target drug levels are listed in Table 43-3. Some drugs may peak earlier than 2 hours, and sampling at 0.5, 1, 2, 4, and 6 hours initially may be informative to establish individual response. Pharmacokinetic data for INH,³⁹ ethambutol,³⁹ and pyrazinamide⁶⁶ in elephants supports dosage recommendations in the 2003 *Guidelines*,⁶⁴ with the exception of INH (see Table 43-3 footnote). Monthly complete blood counts (CBCs) and serum chemistry profiles are advised to assess general health and to screen for hepatotoxicity.

The anti-TB drugs are strong therapeutic agents, and side effects may occur (see Table 43-3).

PREVENTION

Genetics are a key determinant of disease resistance. However, the role of stress in the pathogenesis of TB in humans was recognized as early as 1919.⁵ Although prompt identification of infected individuals, initiation and completion of appropriate therapy, and ongoing surveillance of the population are critical to the control of TB in elephants, the role of stress should not be overlooked. Adequate husbandry, nutrition, and social well-being may well determine which elephants succumb to disease after exposure and which elephants, once infected, respond to treatment.

ZOONOTIC CONSIDERATIONS

Because *M. tb* is primarily a human pathogen, exposure to infected humans is the most likely source of infection for other humans, and *M. tb* infections in animals have been called an “inverse zoonosis.”⁵⁶ Several reports discuss the zoonotic aspects of elephant TB.^{13,37,41,46,49} After the diagnosis of TB in one U.S. herd, 11 of 22 handlers had positive intradermal test results; 3 were considered recent and 8 considered prior exposures. One handler had active TB with the same RFLP pattern as the infected elephant.⁴¹ This is the only reported case of active human TB associated with elephant contact to date. Two Occupational Safety and Health Administration (OSHA) inspections conducted at different facilities concluded that “employees must be working in close proximity to,

and have more than incidental contact with, an infected elephant for transmission to occur."¹³

PERSONNEL HEALTH AND SAFETY

Annual tuberculin testing is advised for personnel working in contact with elephants. Facilities housing TB-positive elephants should develop specific protocols to protect staff from exposure. Consultation with local health department authorities may be helpful.

Respiratory protective equipment should be available during all elephant necropsy procedures and is mandatory for personnel contacting elephants with an unknown, suspect, or positive TB test history. Respiratory protective devices should meet the U.S. Centers for Disease Control and Prevention (CDC) criteria, which include the use of properly fitted, disposable, particulate filter respirators (N-, R-, or P-95, -99, or -100) or positive-air pressure respirators (PAPRs) with high-efficiency filters. The reader is encouraged to consult CDC and OSHA guidelines at the following websites:

OSHA TB standards and rules:

<http://www.osha.gov/SLTC/tuberculosis/standards.html>

CDC *Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings* (draft), 2005:

http://www.cdc.gov/nchstp/tb/Federal_Register/New_Guidelines/TBICGuidelines.pdf

CONSIDERATIONS FOR WILD POPULATIONS

Tuberculosis has not yet been reported in wild elephants. Undoubtedly the potential exists for this occurrence, and the lack of reporting may only reflect a lack of surveillance. Tuberculosis has been reported in captive elephants in several Asian countries (see references cited earlier in Frequency and Distribution), which generally have high rates of human infection and closer public contact with captive elephants. In some countries it is common for wild and captive elephants to intermingle, posing perhaps the greatest risk for disease transfer to wild elephants.

The first report of the introduction of a primary human pathogen into free-ranging wildlife has already occurred. The introduction of *M. tb* to free-ranging banded mongooses (*Mungos mungo*) in Botswana and suricates (*Suricata suricatta*) in South Africa² suggests

that free-ranging African as well as Asian elephants may be at risk.

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CHAPTER 44

Neonatal Elephant Mortality

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The mortality rate of captive elephant calves in North America and Europe has been discussed with growing concern in a number of publications. A survey conducted in 1996 revealed there were significantly higher stillbirths and infant mortality rates in Asian elephants (*Elephas maximus*) living in European and North American institutions than in Asian institutions.¹⁹ Evidence also indicates that the North American Asian elephant population is not self-sustaining.¹² The North American African elephant (*Loxodonta africana*) population statistics are somewhat more encouraging, but this population is also not self-sustaining and will decrease rapidly over the next 50 years without improvements in infant survivability and improved reproduction rates.¹²

The most significant factors affecting captive elephant perinatal and juvenile calf mortality rates have been multifactorial dystocias and stillbirths, herpesvirus infections, maternal rejection, trauma, poor survivability in hand-reared calves, infections, and gastrointestinal disorders.

STILLBIRTHS AND DYSTOCIAS

An overview of the captive birth data reveals a distinct increase in the percentage of dystocias and stillbirths in the older age groups of both Asian and African elephants, primarily in older nulliparous females. Historically, a high percentage of stillbirths has been reported in African elephants, but since 2003, eight viable calves have been born of every nine pregnancies. Still, to date, pregnancies in captive nulliparous African elephants in North America over age 24 years have resulted only in dystocias or stillbirths; just one live viable calf was born to a captive 29-year-old multiparous African elephant.¹¹ There have been no primiparous pregnancies resulting in a live calf in Asian elephants over age 31; however, there have been over seven viable calves born to multiparous members of that age group in North America.⁷

Since 1972, 11 cases of fatal elephant dystocia have been described in the literature.⁶ Eight of these animals were older than 25 years at the time of their dystocia, and all but two were nulliparous at the time of their conception.

The etiology of each dystocia was not always determined, although overweight and physically unfit cows may be at greater risk.¹⁹ Pelvic fusion in older females, uterine abnormalities, and hormonal fluctuations could also be contributing factors. Fetuses that are too large or malpositioned may also result in a dystocia. Fetal death could be either a cause or a result of the dystocia.

In some cases the cause of the dystocia or the stillbirth has been determined. Salmonellosis in two pregnant African elephants at one facility resulted in the death and expulsion of both full-term fetuses.³ Herpesvirus has been found in two stillborn Asian elephant fetuses, suggesting this virus could play a role in the etiology of other stillborn or dystocia cases.

There has been one confirmed case of arthrogryposis of an African elephant fetus causing stiffness and angular deformities in the limbs, which in turn was the likely etiology of that dystocia,⁵ as well as a second suspected case of an arthrogryposis-induced dystocia. *Arthrogryposis* is a growth deformity causing ankylosis of the limbs and has been described in cattle, sheep, and goats. Etiologies of arthrogryposis in other species include genetic predisposition, exposure to Akabane or bluetongue virus, or the ingestion of teratogens containing anagryne or piperidine, found in plants in the Fabaceae family, which includes lupine. There was lupine growing near the elephant exhibit in the one confirmed case of fetal arthrogryposis, although it is not known if the elephant ingested lupine during its pregnancy.

Twinning has been another cause of stillbirths, and although most twins do not survive, one twin of a set was born alive and survived to adulthood.

A cowpox virus infection was responsible for one stillbirth of an Asian elephant.²¹

CONGENITAL ABNORMALITIES

Congenital abnormalities have been described in newborn calves, with one Asian calf exhibiting multiple cardiac congenital anomalies.¹⁸ Umbilical hernias have been described in elephants, although they may not cause serious complications. A 2-week-old Asian elephant calf was diagnosed with an umbilical hernia that was successfully corrected surgically.¹

VIRAL DISEASES

To date, 22 cases of elephant endotheliotropic herpesvirus (EEHV) have been diagnosed in calves 5 years old or younger in Europe and North America.¹⁵ (see Chapter 42). Of those, six were treated with famciclovir, and three survived. Also, EEHV was found in two stillborn fetuses and a 1-day-old calf.

MATERNAL REJECTION AND AGGRESSION

Historically, there has been a high prevalence of maternal rejection, trauma, and infanticide in Western zoos and circuses, which has been correlated to a lack of close contact of the dam with an older female.¹⁹ The problem was created when elephants were first imported into Western facilities as juveniles and were placed together with other unrelated elephants, forming artificial herds of similarly aged young animals. In many cases the first calf born to these reproductively naive females was their first-ever encounter with a calf. They often responded to their first calf as if frightened by it. Now that captive breeding is meeting with greater success and herd experience with calves is more commonplace, maternal rejection or trauma is less frequent and is now generally seen only in inexperienced herds.

Because of the documented instances of maternal rejection or aggression toward newborn calves, most institutions have a policy of immediately pulling newborns from their dam. This allows a thorough veterinary examination to be conducted and allows for controlled introductions of the calf back to the dam.

The birth of the first calf at one institution caused the dam to become frantic, setting off the elephants in the barn to vocalize and trumpet, resulting in an excited environment. The calf was immediately pulled away from the dam. Each time the keepers tried to reintroduce the calf, the dam responded by becoming

frightened and aggressive. With a rope and a harness attached, the calf was gradually introduced to the dam, which was secured with leg restraints. This system allowed trusted keepers to carefully bring the calf close to the dam, allowed them to approach her with the calf from both the side and the front of her body, and allowed the calf to nurse safely from the dam. Introductions were attempted numerous times each day. By the fourth day, the dam accepted the calf and successfully reared it. As more females at that facility gained experience observing and interacting with the calves and the birthing process, subsequent births have been quiet and uneventful.¹⁰

At another facility it took 12 days of steady encouragement to reunite a calf with its dam. During that time the cow was secured with leg restraints whenever the keepers brought in the calf to nurse. Nursing took place every 1 to 2 hours during the day, and the calf was offered a specialized formula at night. The calf was housed within view of the cow but kept at a safe distance. The cow finally accepted the calf, and the staff continued a 24-hour watch for another 2 weeks. Once accepted, this calf was also successfully mother-reared.^{13,14}

As elephant herds grow and adult and juvenile females gain more experience with calves in their herd, some elephant managers may be more comfortable leaving their experienced dams alone during the birthing process. There is evidence that cows with experience around calves of other elephants have fewer tendencies toward rejecting or harming their own calves. Each case is different, and all options must be evaluated carefully.

Dams may be more relaxed if they are gradually habituated to more people and equipment in their birthing area in the weeks to months before the event. Likewise, calves may be rejected because the dam's environment or routine has radically changed in the weeks and especially the days before parturition.

TRAUMA

Elephant calves may incur fatal traumatic injuries from other elephants in the herd or from elements of poor exhibit design. Many exhibits were designed for adult elephants and may have moats or enrichment furnishings that could be inappropriate or dangerous to calves. Exhibits should be examined carefully for spacing in fencing where a calf could become lodged. Methods of preventing calves from falling into moats or other hazards should be applied before calves are allowed into those enclosures.

HAND-REARED CALVES

Hand-reared elephant calves have experienced a high mortality rate in North American and European facilities. The Species Survival Plan (SSP) for African elephants strongly recommends that calves *not* be hand-reared, but rather encourages managers to reintroduce the calves to their dams if at all possible.

Some of the more common causes of mortality in hand-reared calves are complications resulting from failure of passive transfer (FPT) of immunoglobulins, diarrhea caused by inappropriate formula, diarrhea from infectious causes, sepsis, nutritional malabsorption, and metabolic bone disease. Managers of some calves that sustained fractures suggestive of metabolic bone disease agreed that the calves seemed to have consistently loose-to-watery stool and speculated that absorption of nutrients may have been a problem. Access to adequate natural sunlight could be another factor in the cause of this disease.⁴ It is possible the nutritional requirements of calves are not being met when using artificial formulas, suggesting further study is needed in this area. The quandary remains that few hand-reared elephant calves are available for study, and each is a precious member of a dwindling population.

INFECTIOUS DISEASES AND SEPSIS

There has been one reported case of a fatal umbilical infection in an elephant calf.⁹ Serious infections arising from the umbilicus are more likely in immunocompromised calves that may have FPT or in calves stressed for other reasons. Pneumonia does not seem to be a common problem in elephant calves, although it has been a complicating factor in premature calves. Some calves have died from sepsis caused by medical or surgical interventions or traumatic injuries.

GASTROINTESTINAL DISORDERS

A few cases of gastrointestinal impaction have been reported in elephant calves. In one case, surgery was performed on a 3-year-old Asian calf and a sand impaction successfully removed. The calf survived the surgery but died 2 weeks later from complications not associated with the surgery.¹³ Enteritis, volvulus, and torsions have also been causes of mortality, as well as clostridial diseases and salmonellosis.

MEANS OF IMPROVING SURVIVABILITY OF ELEPHANT CALVES AND FUTURE RESEARCH

Survivability of elephant calves is beginning to improve in North America in part because elephant cows have gained more experience and exposure to their calves. Likewise, as more calves survive, nulliparous elephants have a greater opportunity to become familiar with them, which may lead to a decrease in rejection and aggression toward their own future calves.

Although the overall statistics suggest an alarming number of perinatal and infant deaths in captive elephants, there is a recent positive trend of successful births in both Asian and African species. This trend is in part a result of advancements in artificial insemination (AI) technology, which is now responsible for about 50% of elephant pregnancies. AI allows socially stable herds of females to be established, because cows no longer need to be shipped to appropriate bulls for breeding purposes.

Preparation for a birth may be critical to the calf's survival, and most facilities with active breeding programs have detailed birth protocols. One such protocol includes a prepartum preparation list, including habituating the pregnant elephant to leg restraints and training for ultrasound procedures, teat manipulation, and vulva/vestibule palpation, as determined safe by the elephant management team. Cows are also trained to perform a "step" behavior, in which they hold a front leg back to simulate a nursing posture. They are also habituated to the presence of a scale and other equipment in their stall while they are secured with leg restraints, as well as the inclusion of a nursing platform in the event the calf is too small to nurse without it.

Lists of equipment are developed, including block and tackle, hoists, a harness for the calf, slings and belly bands, ropes and lights, and specific veterinary equipment and drugs, and the equipment and supplies are obtained well before the birth of the calf. Plasma is collected from the dam and possibly other elephants before parturition and is frozen to be readily available if needed. Equine hyperimmune plasma has also been used with success for cases of FPT, sepsis, hypoproteinemia, and fluid supplementation. "Birth watch" protocols, as well as neonate and dam evaluation protocols, staff assignments, and other pertinent information, are available to all involved with the process.² Other measures to improve the overall success of elephant reproduction include guidelines developed for veterinary assistance during the reproductive process in elephants.¹⁷

Conditioning pregnant females through exercise and diet and a careful study of the appropriate nutrition for breeding elephants may in turn improve calf survivability. Research to establish data on thermal comfort zones for elephant calves may reveal other necessary husbandry adjustments. With recent advances in veterinary medicine, and as new drugs and treatment protocols emerge, management of cases of EEHV may meet with more success. Success rates of surgical procedures in calves have improved, and successful abdominal procedures have been performed. Newer techniques are being developed, such as vestibulotomies and fetotomies, and show promise in solving dystocias,^{6,16} and although the calf may not survive, at least the cow might be spared. Most managers will likely try to reunite newborn calves with their dams, but hand-rearing techniques and formulas may also improve over time.

The future success of elephant reproduction in North America is encouraging, and the recent improvement is the result of the cooperative efforts of the many institutions and talented individuals dedicated to this goal. Symposia have been organized to help solve the problems facing the survivability of elephant calves, such as the International Elephant Endotheliotropic Herpes Virus Workshop in 2005. This symposium brought together world experts to try to improve methods to diagnose, treat, and prevent EEHV, the primary killer of Asian elephants today. Veterinarians and elephant managers worldwide have continued to contribute their knowledge at symposia and workshops and in the literature to help ensure the success of the reproductive efforts for captive African and Asian elephants.

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Health Care and Management of Working Elephants in Myanmar (Burma)

SHARON L. DEEM AND WAN HTUN

The conservation of Asian elephants (*Elephas maximus*) is fraught with numerous challenges.^{3,12,20} The most serious threats to elephants in Asia are habitat loss, poaching, and conflict with humans.

The global Asian elephant population estimates are 35,000 to 50,000 elephants.²² However, a recent evaluation suggested this estimate is based on a “crude guess” that has been accepted largely unchanged for a quarter century, despite great losses in suitable elephant habitat.³ Currently, Asian elephants are scattered in isolated populations across 13 range states.^{3,14} Myanmar (Burma) is believed to contain the largest captive elephant population (~6000)²² and one of the largest wild populations, with 4000 to 6000 free-ranging elephants.^{3,16} However, more recent discussions among Myanmar elephant experts indicate that estimates for wild elephant populations may be inflated and that free-ranging elephants may now count fewer than 2000.¹³

Little is known about the health status of, and disease threats to, free-ranging and working elephants in Asia despite recent advances in elephant medicine and care.⁹ Diseases of great concern for elephant health include elephant endotheliotropic herpesvirus (EEHV)¹⁹ and tuberculosis (TB),⁸ both of which may significantly affect elephants in Asia. However, studies to determine the prevalence of these diseases in Asia are lacking. Prevalence of TB in Asia’s captive and wild elephant populations, as well as in caretaker populations, should be one of the highest priorities because of the importance of this zoonotic disease not only to wildlife, but also to human populations throughout Asia.

A number of publications address elephant health and disease threats associated with working conditions in Myanmar.* To maximize the benefit of these

resources, it is critical that epidemiologic data on morbidity and mortality of working and free-ranging elephants in Myanmar be collected and included in a systematic manner. These data will help direct future preventive medicine programs for captive elephants and will warn of disease threats to free-ranging elephants, both of which are necessary to ensure the long-term conservation of elephants in Myanmar.

HISTORICAL CONSIDERATIONS

Elephants have been used in Myanmar for centuries as working animals,¹² but their use probably increased dramatically with the large-scale commercialization of forestry after British colonization.²¹ At the start of the twentieth century the number of working elephants used in the logging industry may have been as high as 10,000.^{12,17} In addition to their significant economic role in Myanmar, elephants also have a high cultural and historical value. They most likely have generated considerable environmental value, although this is difficult to quantify. At present, Myanmar has some of the most extensive tracts of forest remaining in Asia,¹⁴ most likely as a direct result of the use of elephants in the timber industry, rather than more destructive, mechanized logging practices. Thus, the working elephants themselves may be a key to land health, which ensures their free-ranging counterparts have habitat for survival.

There are about 6000 working elephants in Myanmar; about half of these are owned by the Myanmar Timber Enterprise (MTE), and half are captive elephants privately owned.²³ In captivity, mortality rates are higher than birth rates, and the working population is maintained by supplementing it with elephants captured from the wild.^{4,15,16} Most working elephants are wild caught, and of the few who are captive born, many are

*References 1, 6, 7, 10, 11, 12, 18.

believed to be sired by wild bulls. There is evidence that continued off-take of wild elephants has been reducing the remaining wild populations of Myanmar.¹⁵ Captive elephants live and work in Myanmar's forests close to the remaining wild herds. This intimate contact may exacerbate the threat to wild elephants by increasing the transmission of disease agents between these two populations. The conservation of wild elephants in Myanmar therefore requires significant improvements in the care and management of captive populations.

Throughout Myanmar, the elephants owned by MTE receive veterinary care from the veterinarians and veterinary assistants employed by the company.²³ Currently, MTE employs approximately 25 veterinarians and 34 veterinary assistants, who provide the health care for the approximately 3000 MTE working elephants (Figure 45-1).

The noninfectious diseases typically encountered by veterinary staff are related to the elephants' working and living conditions. These diseases include traumatic injuries such as wounds, abscesses, and fractures/sprains; eye lesions; foot problems; and gastrointestinal abnormalities resulting from poor-quality food.^{1,5,11} Ectoparasites and endoparasites are a common problem for working elephants, sometimes associated with high morbidity and mortality. Infectious diseases, including anthrax, rabies, tetanus, TB, hemorrhagic septicemia, and foot-and-mouth disease, are also of concern for the captive population.^{1,11}

Although veterinary care has been provided to the working elephants for decades, access to veterinary supplies and laboratory capabilities currently are limited in Myanmar. Most veterinary practices are based on the extensive field experience of lead MTE veterinarians, many of whom must rely on outdated published works.^{6,7,18} It is important now that Myanmar's veterinarians benefit from the extensive advances made worldwide in elephant care over recent decades. Reciprocally, MTE veterinarians may make important contributions to the care and management of elephants worldwide by sharing their extensive practical knowledge and field skills.

WORKING ELEPHANT HEALTH CARE AND MANAGEMENT PROGRAM

Researchers from the Smithsonian National Zoological Park (SNZP) and MTE veterinarians have collaborated on elephant care and management issues in Myanmar for several years. Recently, we jointly initiated a care and management workshop, including SNZP and MTE staff, to determine important needs for the future care and management of captive elephants. SNZP and MTE also have collaborated on ecologic studies on wild elephants using satellite-tracking technology.^{13,24} Both these activities have benefited greatly from MTE's extensive veterinary care program, which covers most of the past century^{6,7,11,18} and continues today.

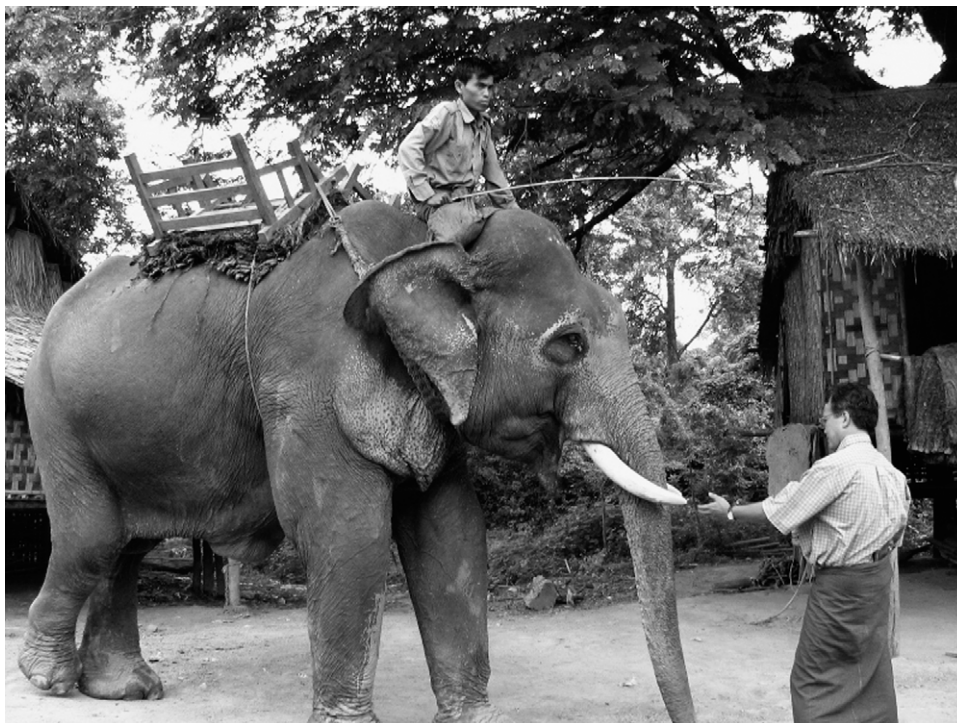


Fig 45-1 Physical examination of working elephant conducted by Myanmar Timber Enterprise (MTE) veterinarian. (See Color Plate 45-1.)

Box 45-1

Major Goals of Working Elephant Health Care and Management Program (WEHCMP) in Myanmar

1. Develop a comprehensive bibliography of all published information on the health and management of Myanmar elephants.
2. Conduct an information exchange and training workshop on elephant management and veterinary care.
3. Perform an epidemiologic evaluation of records available on the historical and current working elephant population.
4. Develop protocols for the Myanmar Timber Enterprise (MTE) veterinarians to collect samples for reproductive, genetic, and health status assessments.
5. Analyze samples and provide data to MTE veterinarians to improve husbandry, preventive care, and treatment of working elephants.
6. Gather ecologic, genetic, and health data on the free-ranging elephants of Myanmar.

Based on the newly initiated training workshop, we hope to expand to a Working Elephant Health Care and Management Program (WEHCMP) that will comprise researchers from many disciplines, including veterinarians, a reproductive physiologist, a geneticist, and a number of ecologists. This program will be directed at addressing the causes of low birth rate and high morbidity and mortality of the captive elephants. Additionally, the program is designed to gain a better understanding of the link between the captive and wild elephants using health indices, genetic and reproductive assessments, and global positioning system (GPS)-tracking technology.

The overall objectives of this new program are (1) to develop long-term captive population management strategies to reduce morbidity and mortality and increase births in the working timber elephants, (2) to stop the continued off-take of animals from the wild that supplement the captive herds, and (3) to minimize health threats to the wild population. To accomplish these overall objectives, the WEHCMP has six major goals⁵ (Box 45-1). The following eight steps will be used to achieve these goals:

1. Determine causes and rates of morbidity and mortality of captive MTE elephants by standardized antemortem diagnostic testing, retrospective and prospective epidemiologic analyses of MTE medical records, and necropsies.
2. Determine causes of low rates of reproduction in captivity.

3. Develop a genetic profile of the captive herds.
4. Develop a protocol to assess "oozie" (mahout) expertise in concert with endocrine and health assessments.
5. Develop small-population viability models to assess how current mortality affects long-term survival of the captive population and what supplementation from the wild is needed for short-term and long-term sustainability.
6. Use population viability models to demonstrate how supplementation from the wild will negatively affect that population.
7. Obtain baseline health parameter data on free-ranging elephants.
8. Quantify habitat and space use using GPS and satellite tracking of captive and wild elephants.

PRELIMINARY PROGRAM ACCOMPLISHMENTS

During an initial visit in 2004, researchers determined that the annual mortality rate for MTE working elephants was 2.4% (66/2750) in 2003. Deaths occurred in all age groups (>18 yr, 40 deaths; 4-17 yr, 11; <4 yr, 15) and included preventable diseases (e.g., poor nutrition, heat stroke, diarrhea, dystocia, infectious, parasitic). Additionally, visual physical examinations were performed on 22 elephants maintained in one of the working camps, and fecal samples were collected and analyzed for endoparasites, genetic profiles, and cortisol levels. On physical examination the most frequent lesions seen were wounds of varying severity on most of the elephants. Three of the 22 elephants (14%) had eye lesions (Figure 45-2), and 4.5% (1/22) had ventral edema of unknown etiology. Fecal sedimentation results revealed a variety of endoparasites, including *Strongylus* spp. (64%), *Triplumaria* spp. (82%), unidentified ciliates (73%), unidentified protozoans (9%), trematodes (4.5%), and mite eggs (9%). Based on genetic analyses, the 22 elephants at this working camp appear to represent a number of family groups from different areas in Myanmar. The mean cortisol level (12 ng/g) in these elephants was lower than that determined for captive Asian elephants in North America.

During this initial visit the SNZP researchers also established a working relationship with the veterinary staff of MTE and at the Yangon Zoo (many of whom provide veterinary care for working elephants). Medical advice was provided for a number of the working elephants, as well as for an orphaned elephant calf and a variety of other species housed at the zoo. After this initial visit, SNZP secured a number of donations



Fig 45-2 Eye lesions, thought to be traumatic in nature, are seen in many working elephants and said to be associated with periorbital lice infestations in some of the animals. (See Color Plate 45-2.)

of much-needed veterinary books and journals for in-country veterinarians.

At the information exchange and training workshop in 2006, the SNZP veterinarian, reproductive physiologist, and conservation biologists discussed elephant medicine at North American facilities of the Association of Zoos and Aquariums (AZA), veterinary contributions to wild and captive elephant conservation, epidemiologic techniques, reproduction in elephants, conservation status of Asian elephants, and “oozie” (mahout)–elephant relationships. MTE veterinarians also presented challenging clinical cases illustrating diseases of working elephants in Myanmar. This information exchange helps the WEHCMP collaborators establish priority needs for the working elephants of Myanmar. The exchange also included distribution of recent literature (e.g., books, reprints) on elephant care, conservation, and medicine. Both in a classroom and in the field, the MTE veterinarians were provided with the equipment and technical skills to perform field diagnostic testing (e.g., fecal parasite determinations, blood testing for hemoparasites, packed cell volume, total solids, white blood cell counts), epidemiologic techniques for future record-keeping advances, and new veterinary treatments to integrate into those already practiced in Myanmar. These capabilities should result in improved health care for the captive elephants and a much larger, more representative sample size for general health, genetic, cortisol, and parasite studies.

CONCLUSION

The collaboration between SNZP and MTE to develop the WEHCMP was originally proposed based on the hypothesis that reducing morbidity and mortality in the working elephants of Myanmar will decrease the need for live capture and the risk of infectious disease agent transmission to wild elephants. The success of this developing program will become evident in the years ahead as researchers monitor morbidity and mortality rates and quantify the off-take of wild elephants to supplement the working elephants. High-quality health care for the working elephants will ensure a healthy working population and the conservation of the remaining free-ranging elephants of Myanmar. The collaborative nature of this program, integrating in-country experts with a multidisciplinary team of international researchers, is its strength. All members are learning from one another and striving to improve the veterinary care of working elephants and to ensure the long-term conservation of elephants in Myanmar.

The conservation issues facing the elephants of Myanmar are an excellent example of the fine line that exists between captive and wild animal management, particularly as it relates to health. The working elephants live with their oozies, who may expose them to diseases such as TB (Figure 45-3). Captive and wild elephants are regularly in direct and indirect contact in the forests, where they forage and live during non-working hours, and thus they too may share disease agents.

The use of working elephants for the extraction of valuable timber provides new strategies for the conservation of elephants and forests. “Elephant logging” is less damaging than machine-operated timber harvesting, which tends to create large roads and unnecessarily damage the understory, soils, and streams.² However, the potential advantages to elephant conservation are offset by the continued need to capture wild elephants to replace those lost as a result of poor management and care.

Minimizing the off-take of elephants from the wild and decreasing disease risks to the wild elephants are imperative. The program outlined in this chapter is one example of how veterinary care for captive animals provides not only health care for a few individuals, but also an array of benefits for their free-ranging counterparts, as well as the humans who care for them. As contact between wild and domestic animals and humans becomes increasingly common, programs such as the WEHCMP will be essential for endangered species’ conservation throughout the world.



Fig 45-3 "Oozies" and elephants live in close contact and thus may share a variety of zoonotic diseases. (See Color Plate 45-3.)

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Color Plate 45-1 Physical examination of working elephant conducted by Myanmar Timber Enterprise (MTE) veterinarian. (For text mention, see Chapter 45, p. 370.)



Color Plate 45-2 Eye lesions, thought to be traumatic in nature, are seen in many working elephants and said to be associated with periocular lice infestations in some of the animals. (For text mention, see Chapter 45, p. 372.)



Color Plate 45-3 "Oozies" and elephants live in close contact and thus may share a variety of zoonotic diseases. (For text mention, see Chapter 45, p. 373.)

CHAPTER 46

Camelids Are Not Ruminants

MURRAY E. FOWLER

WHY BE CONCERNED?

The risk of emerging, reemerging, foreign, and intentionally introduced animal disease is real, and many perceive this as a growing problem. The understanding of animal-human pathogen relationships relies on scientific information about various species and populations of animals. National regulatory statutes that provide disease protection between animals and humans must be current and must utilize up-to-date scientific data to protect human health and our food supply while not jeopardizing or overregulating any one animal.

In the United States, the U.S. Department of Agriculture (USDA), the Department of the Interior (DOI), and the Department of Homeland Security (DHS) are the federal agencies tasked with protecting the nation's wildlife, livestock industries, companion animals, human population, and the food supply from disease. The need for regulations has become more acute with the advent of modern transportation systems that allow movement of animals from any part of the world in a matter of hours. Also, our animal and human populations, along with the U.S. food supply, will now and always need to be protected against the threat of intentionally introduced animal and human disease because bioterrorism will remain a threat.

In a late December 2003 news conference, then-Secretary of Agriculture Ann Veneman made the following statement while talking about a case of bovine spongiform encephalopathy that had been diagnosed Dec. 23, 2003, in a cow in the state of Washington: "The USDA has a primary goal of using science as the basis for decisions involving livestock health matters."

It is not clear that all federal regulatory officials always use known *science* to draft regulations and apply them to risk situations. Zoo veterinarians ask only that camelids, which are classified as domestic animals, and other species of captive and free-ranging wild animals be treated fairly, using science-based consensus of understanding that may be incorporated into laws, regulations, policies, and programs. Current scientific

information is the least that these populations of unique animals deserve.

CLASSIFICATION AND EVOLUTION

Camelids are not ruminants taxonomically, physiologically, or behaviorally.^{7,8} Most importantly, from a veterinary standpoint, camelids and ruminants differ in susceptibility to infectious and parasitic diseases. The differences between camelids and ruminants should exclude camelids from being classified as ruminants. Nonetheless, camelids have been placed in various categories, such as "exotic animals," "wild animals," "other livestock species," and "ruminants," by state and federal regulators. Camelids have consistently been subjected to sudden, adverse regulations (some inappropriate) when an emerging disease of livestock appears on the scene.

The closing of the Canadian border to camelids when bovine spongiform encephalitis was diagnosed in a cow in Alberta, Canada, is a case in point. Camelids were classified as ruminants and subjected to all restrictions placed on ruminants. The fact that camelids have never been diagnosed with any of the transmissible spongiform encephalopathies anywhere in the world (and are not ruminants) was not given proper consideration.

When questioned about that action, the response was that ruminants are defined by an "encyclopedia" as animals that chew a cud, are cloven hoofed, and have three- or four-chambered stomachs. Regulators completely disregarded the scientific literature that clearly shows that foregut fermentation, complex multicompartimentalized stomachs, food regurgitation, and rechewing are not limited to "ruminants" but are found in species as diverse as kangaroos and non-human primates.¹³ In kangaroos, regurgitation and rechewing is referred to as *merycism* (Greek, "chewing the cud"). Foregut fermentation and multicompartimented stomachs are also seen in many species,

Box 46-1

Classification of the Artiodactyla

Class—Mammalia

Order—Artiodactyla

Suborder—Suiformes

Family—Hippopotamidae—Hippopotamuses

Family—Suidae—Pigs

Family—Tayassuidae—Peccaries

Suborder—Tylopoda (L., “padded foot”)

Family—Camelidae

Camelus bactrianus ferus—Wild Bactrian camel*C. bactrianus*—Bactrian camel (two humps)*C. dromedarius*—Dromedary camel (one hump)*Lama guanacoe*—Guanaco*L. glama*—Llama*L. (Vicugna) pacos*—Alpaca*Vicugna vicugna*—Vicuña

Suborder—Ruminantia—Ruminants

Family—Tragulidae—Chevrotain, mouse deer

Family—Moschidae—Musk deer

Family—Giraffidae—Giraffe

Family—Cervidae—Deer, elk, caribou

Family—Antilocapridae—Pronghorn

Family—Bovidae—Cattle, bison, antelope, sheep, goats

including the hippopotamus, kangaroo, colobus monkey, and peccary.⁴

Modern paleontologic and taxonomic scientists clearly state that camelids belong in a separate suborder Tylopoda (Latin, “padded foot”) in the order Artiodactyla, which is distinct from the suborder Ruminantia* (Box 46-1).

Camelid evolution began in North America 40 to 50 million years ago in the early Eocene epoch.^{6,7} Separation of the Tylopoda and Ruminantia occurred early in the evolutionary process, when the progenitors of both groups were small goat-sized animals with simple stomachs.³³

Tylopods and ruminants continued to evolve by what is known as *parallel evolution*, which is the development of similarities in separate but related evolutionary lineages through the operation of similar selective factors acting on both lines^{6,7,33} (Figure 46-1).

The Pleistocene epoch was characterized by a series of periods of extreme cold and glaciations in northern North America and Europe. The last glacial retreat occurred about 10,000 years ago, marking the beginning of the Recent epoch.

Asia and Alaska are now separated by the 90-km (56-mile)-wide Bering Strait. However, during the height

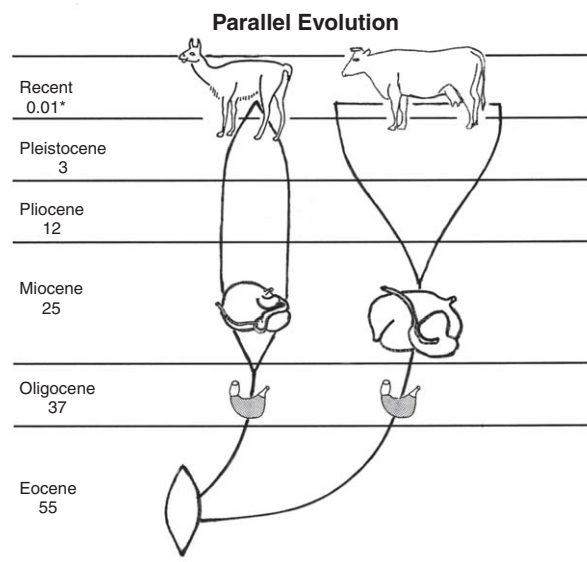


Fig 46-1 Diagram of parallel evolution of Camelidae and Ruminantia.

of one of the early Pleistocene glacial periods, the sea level was lowered sufficiently to expose a wide land bridge. Plant and animal species moved back and forth across this bridge; the camel line of Camelidae migrated from North America into Asia, where the evolutionary process continued and domestication took place.

Progenitors of the South American camelids (SACs) (guanaco, vicuña, llama, and alpaca) migrated to South America at the beginning of the Pleistocene epoch (~3 million years ago), when an open land connection between North and South America developed.⁶ Evolution continued in South America, where llama and alpaca were domesticated.^{9,10,35}

DIFFERENCES BETWEEN CAMELIDS AND RUMINANTS

Anatomic and physiologic differences between camelids and ruminants abound (Table 46-1).

Susceptibility to infectious and parasitic agents is of greater concern. The USDA Animal and Plant Health Inspection Service (APHIS) has stated that camelids should be classified as ruminants because, “regardless of their taxonomic classification, camelids meet the definition of ruminants and are regulated as ruminants based on their susceptibility to ruminant diseases such as foot and mouth disease, tuberculosis (*Mycobacterium bovis*, *M. tuberculosis*, and *M. avium*), brucellosis, Johne’s disease, etc.”¹¹

It is true that there are diseases that camelids, cattle, sheep, and goats all acquire, but a careful appraisal of Tables 46-2 through 46-9 should dispel the myth that

Text continued on page 383

*References 1, 3, 4, 7-10, 15-17, 20-24, 26-29, 32, 35, 36.

Table 46-1

Differences Between Camelids and Ruminants

	South American Camelids	Ruminants
Evolutionary pathways	Diverged 40 million years ago.	Diverged 40 million years ago.
Blood		
Red blood cells	Elliptic and small (6.5 μm).	Round and large (10 μm).
Predominant white blood cell	Neutrophil.	Lymphocyte.
Leukocytes	Up to 22,000.	Up to 12,000.
Blood glucose levels	Higher than ruminants (73-121 mg/dL).	18-65 mg/dL.
Integument		
Horns or antlers	None.	Usually present in male.
Foot	Triangular-shaped toenails and fat pad covered by soft, flexible slipper.	Has hooves and sole.
Upper lip	Split and prehensile.	Not split.
Flank fold	None.	Pronounced.
Musculoskeletal system		
Stance	Modified digitigrades.	Unguligrade ending in a hoof.
Second and third phalanges	Horizontal.	Almost vertical.
Foot	Not cloven.	Cloven.
Dewclaws	None.	Many have dewclaws.
Digestive system		
	Foregut fermenter, with regurgitation, rechewing, and reswallowing.	Same (parallel evolution).
Stomach	Three compartments not homologous with rumen, reticulum, omasum, and abomasum; all compartments have glandular epithelium; stomach motility from caudad to cranial; resistant to bloat.	Four compartments; susceptible to bloat.
Dental formula*	I 1/3, C 1/1, PM 1-2/1-2, M 3/3 \times 2 = 28-32 Vicuña has incisors that continue to erupt.	I 0/3, C 0/1, PM 3/3, M 3/3 \times 2 = 32
Reproduction		
Ovulation	Induced.	Spontaneous.
Estrous cycle	No.	Yes.
Follicular wave cycle	Yes.	No.
Copulation	In prone position.	In standing position.
Placenta	Diffuse and noninvasive.	Cotyledonary.
Epidermal membrane	Surrounding fetus.	None on fetus.
Cartilaginous projection on tip of penis	Yes.	No.
Ejaculation	Prolonged.	Short and intense.
Respiratory system		
Soft palate	Elongated; primarily a nasal breather.	Short; nasal or oral breather.

*I, incisors; C, canines; PM, premolars; M, molars.

Continued

Table 46-1—cont'd

Differences Between Camelids and Ruminants

	South American Camelids	Ruminants
Urinary system		
Kidney	Smooth and elliptic.	Smooth or lobed.
Suburethral diverticulum	In female at external urethral orifice	None
Dorsal urethral recess	In male at junction of pelvic and penile urethra.	In some species.
Parasites		
Lice	Unique biting and sucking lice.	Lice species different.
Coccidia	<i>Eimeria</i> species (coccidia) are different.	Unique species of coccidia.
Gastrointestinal nematodes	Share some with cattle, sheep, and goats.	Share with camelids.
Infectious diseases		
Tuberculosis	Minimally susceptible.	Highly susceptible.
Bovine brucellosis	No known natural.	Highly susceptible.
Foot-and-mouth disease	Mild susceptibility. Rare clinical disease with other bovine and ovine viral diseases.	Highly susceptible.
Behavior		
	Females do not lick their offspring	Females lick offspring.
	Females do not touch/lick aborted fetuses.	Females investigate dead fetuses.
	Females do not consume the placenta.	Females may consume the placenta.

Table 46-2

Clinical Infectious Diseases of Camelids and Ruminants

Camelids and Ruminants	Ruminants (Not Seen in Camelids)	Camelids (Not Seen in Ruminants)
Contagious ecthyma	Malignant catarrhal fever	Camelpox
Rabies (common to many mammals)	Bovine leukemia	Camel papillomatosis
Foot-and-mouth disease (FMD; occurs in many nonruminants)	Cowpox	<i>Mycoplasma hemolama</i> (Eperythrozoonosis)
Rinderpest (camels)	Pseudorabies	Lama adenoviruses, serotypes 1-6
West Nile virus (WNV) encephalopathy (seen in many mammals and birds)	Bovine papillomatosis	
Fungal diseases (ringworm) (common to many mammals)	Ovine progressive pneumonia	
Tetanus and other clostridial diseases	Sheeppox or goatpox	
Bovine tuberculosis (seen in many nonruminant species)	Balanoposthitis	
Johne's disease	Sheep or goat papillomatosis	
Necrobacillosis	Scrapie	
Streptococcosis (common to many nonruminant species)	Bovine spongiform encephalopathy (BSE)	
Staphylococcosis (common to many nonruminant species)	Chronic wasting disease (CWD) of cervids	
Caprine/ovine brucellosis	Bovine brucellosis	
	Anaplasmosis	

Table 46-3

Infectious Disease Agents Producing Antibody Response, but Rare or No Clinical Disease in Camelids

Agent	Disease
Bovine herpesvirus type 1	Infectious bovine rhinotracheitis
Equine herpesvirus type 1	Equine rhinopneumonitis Retinal degeneration in SACs
Bluetongue/epizootic hemorrhagic disease virus	Bluetongue, epizootic hemorrhagic disease of deer
Rift Valley fever virus (camels)	Rift Valley fever
Rotavirus	Enteritis (diarrhea)
Coronavirus	Enteritis (diarrhea)
Adenovirus	Enteritis (diarrhea)
Encephalomyocarditis virus	Encephalomyocarditis (EMC)
<i>Brucella abortus</i>	Bovine brucellosis
Borna disease virus	Viral encephalitis
Vesicular stomatitis virus	Vesicular stomatitis

SACs, South American camelids.

Table 46-4

Programmed Diseases of Ruminants in United States Compared with Camelids and Other Species

Programmed Diseases of Cattle, Sheep, and Goats	CLINICAL DISEASES IN CAMELIDS			Nonruminant Hosts Developing Natural Disease
	From Natural Transmission	From Experimental Inoculation	Antibody Response in Camelids	
Bovine brucellosis <i>Brucella abortus</i>	None	Yes	Yes	Humans, horses (fistula of withers), carnivores, marine mammals
Bovine tuberculosis <i>Mycobacterium bovis</i>	Yes (rare)	Yes	Yes	Humans, European badger, brush-tailed possum
Chronic wasting disease (CWD) of cervids	None	None	None	None
Scrapie	None	None	Not applicable	None

Table 46-5

Comparison of Ruminant Emergency Conditions, Compared with Camelid and Other Species

Emergency Conditions of Cattle, Sheep, and Goats	CLINICAL DISEASES IN CAMELIDS			Nonruminant Hosts Developing Natural Disease	Nonruminant Hosts, Experimental Disease
	From Natural Transmission	From Experimental Inoculation	Antibody Response in Camelids		
Anthrax	Yes	Not reported	Not reported	Humans, numerous species of mammals	Many
Bovine spongiform encephalopathy (BSE)	No	No	Not applicable	Human, cat, cheetah, lion, tiger, puma	Brain extracts from infected cattle have produced disease in cattle, sheep, pigs, and mice.
Contagious bovine pleuropneumonia (mycoplasmosis)	No	Not reported	Not reported	None	Not reported
Foot-and-mouth disease (FMD)	Yes (rare)	Yes	Yes	Hedgehogs, pigs, peccaries, insectivores, xenarthra, rabbits, squirrel, hyrax, elephant, bears, marsupials	
Hemorrhagic septicemia <i>Pasteurella multocida</i> + other agents	None reported	Not reported	None reported	Broad range of mammals	Wide variety
Malignant catarrhal fever (African)	None reported	Not reported	One llama	Pigs (Norway)	Rabbit
Rift Valley fever	Yes, camel	Not reported	None reported	Human, dog, cat, rodents	Unknown
Rinderpest	Yes, camel	Not reported	Yes	Pig, peccary	Pig, peccary, dog, elephant, hyena, jackal, tiger, vulture, zebra
Vesicular stomatitis	Yes (rare)	Yes	Yes	Horse, pig	Unknown
Contagious agalactia (mycoplasmosis)	None reported	Not reported	Not reported	None	Unknown
Contagious caprine pleuropneumonia (mycoplasmosis)	None reported	Not reported	Not reported	None	Unknown
Heartwater <i>Ehrlichia</i> (formerly <i>Cowdria ruminantium</i>)	None reported	Not reported	Not reported	Numerous vertebrates may be intermediate hosts for <i>Ehrlichia</i>	Unknown
Nairobi sheep disease (tick borne, viral)	None reported	Not reported	Not reported	African field rat	Not successful at experimental transmission
Peste des petits ruminants	None reported	Not reported	Not reported	Not reported	Unknown
Pulmonary adenomatosis	None reported	Not reported	Not reported	Not reported	Unknown

Table 46-6

Comparison of Regulated Infectious and Parasitic Diseases of Ruminants Compared with Camelids and Other Species

Regulated Diseases of Cattle, Sheep, and Goats	CLINICAL DISEASES IN CAMELIDS			Nonruminant Hosts Developing Natural Disease
	From Natural Transmission	From Experimental Inoculation	Antibody Response in Camelids	
Rabies	Yes	Not reported	Yes	Most species of mammals
Bovine brucellosis <i>Brucella abortus</i>	None	Yes	Yes	Human, horse, carnivore, marine mammals
Bovine tuberculosis <i>Mycobacterium bovis</i>	Yes (rare)	Yes	Yes	Human, European badger, brush-tailed possum
Bovine scabies (mange) <i>Sarcoptes scabiei</i> , <i>Psoroptes ovis</i>	Yes	Not applicable	Not applicable	Many mammal species
Trichomoniasis <i>Tritrichomonas fetus</i>	None reported	Not reported	Not applicable	Unknown
Caprine/ovine brucellosis <i>Brucella melitensis</i>	Yes	Not reported	Yes, may cross react with bovine brucellosis	Human
Scrapie	None	Not reported	Not applicable	None
Sheep/goat scabies <i>Psoroptes ovis</i>	Yes	Not applicable	Not applicable	Unknowns

Table 46-7

Monitored Diseases of Ruminants in United States Compared with Camelids and Other Species

Monitored Diseases of Cattle, Sheep, and Goats	CLINICAL DISEASES IN CAMELIDS			Nonruminant Hosts, Developing Natural Disease	Nonruminant Hosts, Experimental Disease
	From Natural Transmission	From Experimental Inoculation	Antibody Response in Camelids		
Avian tuberculosis	Yes	None reported	Yes	Many species of birds and mammals; swine; humans	Unknown
Anaplasmosis	No	Yes	Yes	None	Unsuccessful attempts
Bluetongue	Yes, but with questions	Not reported	Yes	None	Raccoon, opossum, hares
Bovine leukosis, viral	None reported	Not reported	Not reported	None	Unknown
Johne's disease	Yes	Not reported	Yes	Rabbits, nonhuman primates	Unknown
Malignant catarrhal fever (North America)	None reported	Not reported	Not reported	None	Unknown
Bovine cysticercosis	None reported	Not reported	Not applicable	Human <i>Taenia saginata</i>	None reported
Infectious bovine rhinotracheitis	None reported	Not reported	Yes	None reported	Unknown
Bovine genital campylobacteriosis (vibriosis)	None reported	Not reported	Not reported	None reported	None reported

Continued

Table 46-7—cont'd

Monitored Diseases of Ruminants in United States Compared with Camelids and Other Species

Monitored Diseases of Cattle, Sheep, and Goats	CLINICAL DISEASES IN CAMELIDS				
	From Natural Transmission	From Experimental Inoculation	Antibody Response in Camelids	Nonruminant Hosts, Developing Natural Disease	Nonruminant Hosts, Experimental Disease
Echinococcosis	Yes	Not reported	Not applicable	Humans, carnivores	Unknown
Leptospirosis	Yes	Not reported	Yes	Numerous mammals	Rodents and rabbits
Ovine progressive pneumonia (Maedi-Visna)	None reported	Not reported	Not reported	None reported	None reported
Q fever	None reported	Not reported	Not reported	Humans and many other species	None reported
Caprine arthritis/encephalitis	None reported	Not reported	Not reported	None reported	Unknown
Ovine chlamydiosis <i>Chlamydia psittaci</i>	None reported	Not reported	Not reported	Birds, humans, koala	Numerous species
Ovine epididymitis <i>Brucella ovis</i>	None reported	Not reported	Not reported	None	None

Table 46-8

Comparison of Regulated Parasitic Diseases of Cattle, Sheep, and Goats with Camelids

Regulated Parasitic Diseases of Cattle and Sheep	Etiology	Status in Camelids	Intermediate Hosts	Location in Host
Screwworm myiasis	<i>Cochliomyia hominivorax</i> or <i>Chrysomya bezziana</i>	All animals, including camelids, may become infested with screwworms.	None	Wounds, necrotic tissue
African trypanosomiasis (surra)	<i>Trypanosoma evansi</i>	Important disease of camels; may involve other species of <i>Trypanosoma</i> . SACs also infected.	Blood-sucking flies (tabanids, <i>Stomoxys</i>), tsetse flies, and other	Blood
Bovine babesiosis (piroplasmosis)	<i>Babesia bovis</i>	No verified reports in either camels or SACs	Ticks	Blood
Theileriosis (East Coast fever, corridor disease)	<i>Theileria</i> spp.	No verified reports in either camels or SACs	Ticks	Blood
Cattle scabies (multiple types)	<i>Sarcoptes scabiei</i> , <i>Psoroptes ovis</i>	Both may infest camelids.	None, direct contact	Skin
Sheep scabies	<i>Psoroptes ovis</i>	Yes	None, direct contact	Skin
Echinococcosis (hydatid disease)	<i>Echinococcus granulosum</i>	Many species, including camelids	Carnivore is primary host; herbivores are intermediate host.	Variable, but liver and lungs common

SACs, South American camelids.

Table 46-9

Comparison of Selected Parasitic Diseases of Ruminants with Camelids

Parasitic Diseases of Ruminants	Etiology in Ruminants	Status in Camelids	Etiology in Camelids	Location in Host	Comments
Pediculosis (lice)	Biting lice <i>Damalinia bovis</i> (cattle) <i>Damalinia ovis</i> (sheep) Sucking lice in ruminants (<i>Haematopinus</i> , <i>Linognathus</i> , and <i>Solenopotes</i>)	None of the lice of ruminants infect camelids, or vice versa.	Biting louse of SACs: <i>Damalinia breviceps</i> ; none in camels Sucking lice: <i>Microthoracis</i> spp. (<i>M. cameli</i> , <i>M. mazzai</i> , <i>M. minor</i> , <i>M. praelongiceps</i>)	Skin	Biting lice do not readily respond to ivermectin therapy.
Coccidiosis	<i>Eimeria bovis</i> , <i>E. zuernii</i> , and many other <i>Eimeria</i> spp.	Not a common parasite, and usually only in young animals	<i>Eimeria lamae</i> , <i>E. alpaca</i> , <i>E. punoensis</i> , <i>E. macusaniensis</i> , <i>E. bactriani</i> , <i>E. cameli</i> , <i>E. dromedarii</i> , <i>E. pellerdyi</i>	Small intestine	It is common to find coccidia in feces, but animals should not be treated unless clinical syndrome is severe.
Trichuriasis (whipworms)	<i>Trichuris ovis</i>	Common	<i>Trichuris tenuis</i>	Large intestine	Serious parasite of camelids
Nematodiriasis	<i>Nematodirus</i> spp.	May be a significant parasitism	<i>Nematodirus battus</i> , <i>N. lamae</i>	Small intestine	
Spiculopteragiasis	Does not affect cattle, sheep, or goats in South America	Found only in South America Unique to SACs	<i>Spiculopteragia peruviana</i>	Small intestine	
Graphinemiasis	Does not affect cattle, sheep, or goats in South America	Found only in South America Unique to SACs	<i>Graphinema aucheniae</i>	Small intestine	
Lamanemiasis	Does not affect cattle, sheep, or goats in South America	Llama is secondary host. Primary host is a rodent (viscacha).	<i>Lamanema chavezii</i>	Small intestine	Serious parasite of young alpacas Affects the liver

Data from Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, 1998, Iowa State University Press; Wernery U, Kaaden OR: *Infectious diseases in camelids*, ed 2, Boston, 2002, Blackwell Science; and Bowman DD: *Georgis' parasitology for veterinarians*, ed 8, Philadelphia, 2003, Saunders

SACs, South American camelids.

"Llamas and alpacas are susceptible to all cattle and sheep diseases."³⁴ In fact, they are quite resistant to many regulated ruminant diseases.

Foot-and-mouth disease (FMD) virus is highly contagious in cattle and sheep. When llamas and alpacas were first imported from South America to the United States for the blossoming private llama industry, government officials expressed concern that llamas and alpacas might pose a risk for the introduction of FMD to the United States. The USDA expended con-

siderable experimental effort to determine the risk. It was concluded that llamas and alpacas could be infected by inoculation but did not acquire FMD when cohabiting with infected swine, in contrast with almost 100% of cattle that acquired the infection.^{6,25,30} The virus could not be detected after 14 days postinoculation.

The same could be said for vesicular stomatitis. Only one animal has been diagnosed with the natural disease.² Llamas may be infected experimentally.¹⁴

Bovine tuberculosis (TB) caused by *Mycobacterium bovis* is another concern of government officials. Llamas and alpacas have developed the disease under natural conditions, when cohabiting with infected elk, but have shown resistance to acquiring TB, in contrast to ruminants.³¹

Llamas and alpacas have been experimentally infected with *Brucella abortus*, but the natural disease does not occur in these species.^{12,13}

There are no reports of the transmission of any regulated ruminant disease from camelids to ruminants.

CONCLUSION

Camelids are not ruminants taxonomically, anatomically, physiologically, or behaviorally. Camelids also are not a threat to the livestock industry because they either have total resistance to infection or have minimal susceptibility to the infectious and parasitic diseases of ruminants.

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Uroliths and Gastroenteroliths in Camelids

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Obstruction of the tubular urinary or digestive tract may cause acute colic signs and may be life threatening in any species of animal. Veterinarians are well aware of the syndromes in traditional livestock and companion animals. Similar conditions occur in zoo animals.

Historically, camelids have always been considered zoo animals, but with the advent of the public fancy for llamas and alpacas, the prevalence of uroliths and gastroenteroliths has been more frequently reported. Information gleaned from these cases may be of value to those dealing with camelids in zoos.

CONCRETIONS OF URINARY TRACT

Urinary calculi (*urolithiasis*; uroliths, nephroliths, bladder stones, cystoliths) are formed in either the calices of the kidney or, more often, the urinary bladder. Small uroliths may enter the ureter or urethra and cause partial or complete obstruction of urine flow.* No specific studies on the pathogenesis of urinary calculi formation in camelids have been reported. There are clinical reports of disease caused by the obstruction produced by the calculi. Zoo veterinarians must rely on information extrapolated from studies of cattle, sheep, and goats, which have urine of similar composition (Table 47-1). Box 47-1 lists the composition of urinary calculi recovered from llamas and alpacas at the Veterinary Medical Teaching Hospital, University of California. Others have reported similar findings in camels, vicuña, and guanacos. Urethral obstruction may also occur with nonmineral concretions.

Urinary calculi are formed in males and females equally, but the bore (diameter) of the female urethra generally allows free passage of a calculus that may enter the urethra. Thus, obstructive urolithiasis is rare

in the female. Urolithiasis has been associated with a diet high in concentrated feeds, as often used in zoos. Cattle pastured on grasses containing high levels of silicates may sometimes develop silicate urolithiasis, and presumably, camelids grazing on such pastures may be at risk as well.

A basic understanding of the camelid urethra is required to locate sites of possible obstruction and develop approaches to management. Figure 47-1 is a diagram of the camelid urethra and associated structures. The prostate gland does not surround the pelvic urethra as in carnivores. The pelvic urethra is expansive, but at the reflection around the ischium, only a tiny orifice allows passage of urine beyond this point. The anatomy of this area is further complicated by a dorsal urethral recess that precludes any possibility of passing a catheter into the bladder from the tip of the penis. Whereas the sigmoid flexure is the probable site of the majority of bovine urethral obstructions, this is not the case in camelids. The orifice from the pelvic urethra into the penile urethra is a common site of obstruction; another site is where the penile urethra narrows as it enters the glans penis.

Clinical Signs

Uroliths in the bladder or calices of the kidney rarely cause discomfort, although large and rough-surfaced uroliths may initiate a cystitis. The signs of urethral obstruction caused by calculi vary with the stage of the disorder.⁹ Signs prior to bladder rupture include colic, straining stance to urinate, dribbling urine, blood-tinged urine, anuria, distended bladder, and possible pulsation of the urethra. Signs after bladder rupture are absence of colic, with depression, anorexia, anuria, uroperitoneum, and possible distention of the abdomen and uremia (muscular weakness, dehydration, dyspnea, tremors, uremic breath odor, tachycardia, recumbency, coma, death).²⁴

*References 6, 7-12, 16, 17, 20, 21, 24.

Table 47-1

Characteristics of Normal Urine

Parameter	Camelid	Bovine	Equine	Canine
pH	7.0-8.5, alkaline	Alkaline	Alkaline	Acidic
Specific gravity	1.010-1.048	1.015-1.045	1.020-1.050	1.020-1.045
Color	Clear, yellow to amber	Clear, but becomes cloudy on standing	Cloudy	Clear
Solids	Calcium oxalate; uric acid rarely	Phosphates	Calcium carbonate	Uric acid

Box 47-1

Composition of Urinary Calculi*

Silicon dioxide (SiO₂)—Crystobalite
 Magnesium ammonium phosphate (MgNH₄PO₄ 6H₂O)—Struvite
 Basic calcium phosphate (Ca₅[PO₄]₃[OH])—Apatite
 Calcium carbonate (CaCO₃)—Carbonate
 Uric acid (C₅H₄N₄O₃)—Urate

*Recovered from llamas at the Veterinary Medical Teaching Hospital, University of California.

Uroperitoneum may result from trauma when the bladder is distended or from rupture of the bladder after urethral obstruction. In the llama the immediate response to urine flushing into the abdominal cavity is excruciating pain. Llamas become frenzied and thrash about violently. This initial pain subsides, and the pain associated with a distended bladder disappears. Urine in the abdomen may arise from a single or multiple tears in the bladder wall, but also from seepage through the stretched-thin bladder wall. A ureter may also rupture, but such a condition has not been diagnosed in a camelid.

Diagnosis

Diagnosis is based on assessment of clinical signs, pertinent history, and special diagnostic tests. A differential diagnosis should include any disease condition in which colic is a sign. The major hematologic and serum chemistry changes found in camelids with obstructive urolithiasis include hemoconcentration, elevated blood urea nitrogen (BUN), hypophosphatemia, hypercalcemia, hypermagnesemia, hyperkalemia, hypercreatininemia, and hypochloremia.²⁰ It is not always easy to identify the source of fluid in the abdominal cavity. Urine should have the odor of ammonia, but it may be neces-

sary to heat the fluid to concentrate the odor before it becomes perceptible. The ability of individuals to smell ammonia varies. Abdominal fluid caused by urine has a creatinine concentration greater than 15 mg/dL (normal serum <3 mg/dL). Urine potassium is greater than 185 mmol/L (normal serum <5 mmol/L). The latter is the most important biochemical urine detector.

Urethral catheterization and double-contrast radiography are routinely used to diagnosis urethral calculosis in carnivores. It is impossible to pass a catheter in a camelid male because of the restrictive urethral diameter and the dorsal urethral recess. The value of radiography and ultrasonography has been less than satisfactory. Uroliths that do not contain calcium may be radiolucent, especially when surrounded by an inflammatory reaction.

The narrow urethra may predispose the accumulation of organic debris. A llama developed a swelling of the preputial area that was treated initially as a trauma and later as a cellulitis. At necropsy the urethra had ruptured proximally to an obstruction at the glans penis caused by a plug of nonmineralized organic material. The urethra may also become obstructed by external pressure on the urethra caused by a hematomoma of the corpus cavernosum urethra after penile trauma.

Management

In most species, urethral obstruction caused by uroliths is more common in castrated males, but unfortunately in llamas, breeding males are most frequently the victims. The first task is to relieve the obstruction and restore urine flow if the bladder has not already ruptured.

Traditional therapy for feedlot steers is to perform a urethrostomy and/or penile amputation just ventral to the ischium, bringing the penile stump out through the incision and suturing it in place.²² In breeding male

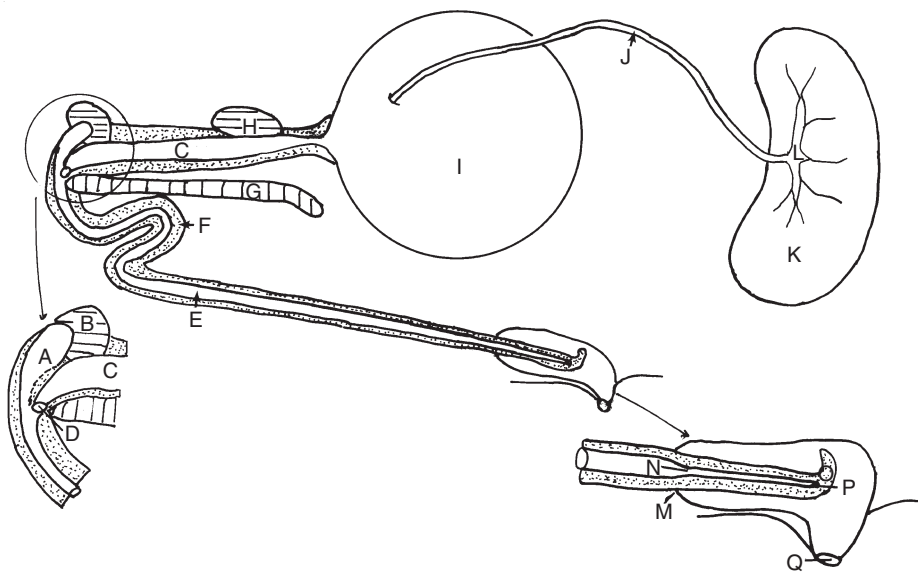


Fig 47-1 Diagram of urinary tract of male camelid. A, Dorsal urethral recess; B, bulbourethral gland; C, pelvic urethra; D, narrowed junction of pelvic and penile urethra; E, penile urethra; F, sigmoid flexure; G, pelvis; H, prostate gland; I, bladder; J, ureter; K, kidney; L, calyx of kidney; M, reflection of prepuce onto penis. This is the beginning of the glans penis; N, narrowing of urethra at origin of glans penis; O, cartilaginous projection at tip of penis; P, urethral orifice; Q, preputial opening.

llamas an attempt is made to localize the obstruction and perform a urethrostomy over the urolith. Unfortunately, the prognosis for a return to breeding is poor to unfavorable. Dietary management in herbivores is not highly successful.

Complications

Potential sequelae of urolithiasis include ruptured bladder, ruptured urethra, urethral stricture caused by trauma from the calculus, urethral stricture caused by scarring after surgical manipulation, chronic cystitis, and recurrence of calculi.

GASTROINTESTINAL CONCRETIONS

In ancient times, people marveled at the “stones” that animals were thought to have swallowed. These stones took on the aura of magic in some cultures and protection against poisons in other cultures. Concretions are technically thought to be mineral in origin, but many consist of combinations of minerals, plant, and even animal tissue (hair).

Concretion derives from a Latin word meaning “to grow together.” *Bezoar* is an ancient word derived from the French word *bézoard*, Arabic *bazahr*, or Persian *padzahr*, all meaning “an antidote.” *Bezoars* were once thought to be an antidote to poison. *Gastrolith* is a stomach stone, *enterolith* an intestinal stone, *phytobezoar* a plant stone, and *trichobezoar* a hair ball.

Poisoning was once a common form of murder, and bezoars were thought to be antidotes to poisons. Royalty tried to protect themselves from poisoning by

keeping bezoars about their persons. Modern investigation has determined that bezoars may in fact neutralize arsenical poison.¹⁵ The Spanish invaders of the Altiplano (Pizzaro, 1532) noticed that the native Incas ascribed magical and curative properties to the small stones that they found in the stomachs of all the South American camelids (SACs). A historian reports that the superstitious Spaniards coveted these stones, which led to the mass slaughter of alpacas and llamas and even the hunting of vicuña and guanacos. These prized stones were made into amulets to protect the wearer from evil and witchcraft. The stones are also used currently in rituals and are placed on tables containing the offerings to the *Pachamama* and the *Apus*. Bezoars are much sought after when an alpaca or llama is killed.¹⁸

Etiology

Most of the concretions found in the stomach or intestine of camelids are the result of precipitation of consecutive layers of mineral matter (phosphates) around a central kernel of plant fiber or seed awn in saccules of the first stomach compartment. Not all these spherical objects are made up of mineral matter; three types of bezoars have been found in the stomach and intestines of camelids: (1) mineral stones (concretions, gastroliths, enteroliths), (2) compacted plant fiber balls (phytobezoars, fecaloliths, impactions), and (3) hair balls (trichobezoars, zootrichobezoars). A fourth type may be a combination of one or more of the other three.

The accumulation of sand in a segment of the intestine may be closely allied to the “stones” and cause similar signs of distress. All these types have been

detected in the digestive tracts of camelids, ruminants, horses, and humans.

Mineral stones are common in the saccules of the camelid stomach.* Plant fiber balls become major intestinal obstructions when they develop in the pellet-forming portion of the spiral colon. The diameter of the intestine decreases by five times in the spiral colon, and any large, firm object may completely occlude passage of normal pellets to the rectum. Cats and rabbits are notorious for developing hair balls because of their habit of licking their fur during grooming. Camelids seldom lick anything, so hair balls are rare, although these have been found in crias that develop the habit of chewing the fiber coat of their mothers. Most often this is observed in crias that have nasal obstruction (choanal atresia) and difficulty breathing while eating normally. Such crias stand with the head and neck extended and chew fiber to satiate hunger.^{2,3}

Sizes of gastroliths vary from 1 mm ($\frac{1}{32}$ inch) in diameter to objects the size and shape of a small to medium chicken egg (2.5-4.0 × 4-5 cm [1.0-1.6 × 1.6-2.0 inches]). The surface of the gastrolith may be smooth or rough. Usually, when a stone is cut through the middle, a kernel of plant fiber may be seen that served as the nucleus for precipitating the mineral from the normal stomach contents. The mechanism that triggers the process is unknown. Concretions may form directly in the intestine of horses and perhaps other species, but in camelids the likely source of intestinal concretions is the stomach.

The saccules of the first compartment of the camelid stomach may be an ideal place for concretions to form. The secretions from the glands of the saccules have been intensively studied by physiologists and nutritionists. An important component of the secretion is *bicarbonate*, which produces a slightly basic reaction. Bicarbonate is also secreted by the salivary glands, and concretions may form in the salivary ducts of horses and people. The basic reaction of the secretion within the sacculus is conducive to the precipitation of phosphates and carbonates, forming concretions over months or years.

The composition of gastrointestinal (GI) enteroliths is more complex than that of uroliths. Preliminary studies show that the mineral composition of GI enteroliths includes various forms of calcium and phosphate. Factors that may contribute to the formation of phosphate concretions in the intestinal tract may include diets high in magnesium or high pH of the ingesta. Formation of trichobezoars may be associated with ingestion of plants that have tiny hairs on the sur-

face of leaves or ingestion of hair (aberrant behavior or pica in crias).

Clinical Signs

Concretions located in the saccules of compartment one (C-1) of the stomach cause no impairment of gastric function, as far as is known. The stones are seen as incidental findings on radiographs of the midventral abdomen or at necropsy. Other concretions may cause obstruction, ulceration, and perforation of the viscus wall. The clinical signs of bezoar obstruction of the stomach or intestine are the same as for any obstruction, including anorexia, cessation of fecal passage, colic, refusal to drink, and depression. Without surgical treatment, the animal may die in 2 to 5 days. A large mineral-encrusted phytotrichobezoar caused obstruction of compartment three (C-3) of the stomach and ultimately killed a zoo llama.¹⁷

Diagnosis

Concretions in C-1 may be seen on a radiograph of the abdomen. Small concretions lying in a segment of the intestine overlaid by C-1 will be obscured. Many digestive tract disorders (impaction of C-3, ulceration of C-3, impaction of intestines, torsion, ileus) produce similar signs. Furthermore, uterine torsion and obstructive urolithiasis produce the same signs. Any animal with colic and depression should be given a thorough examination to rule out digestive tract obstruction.

Hematologic and blood chemistry findings may vary from normal to those of an inflammatory syndrome in the animal with peritonitis or severe gastritis. Dehydration is reflected in an elevated packed cell volume and hyperproteinemia. Evaluation of abdominal fluid obtained by abdominocentesis is a valuable adjunct, particularly in establishing the presence of peritonitis.

At necropsy the obstructing concretion is usually evident, except for phytobezoars located at the reflection of the spiral colon as it begins its centripetal coiling. Such obstructions may be easily overlooked because the intestine is hidden by the fibrous tissue that binds the spiral colon together.

Therapy

Concretions in C-1 require no therapy. If the concretion obstructs the intestine, it must be removed

*References 1, 2, 3, 6, 14, 17, 18, 25.

surgically. Recommended treatment for sand impaction is repeated doses of mineral oil or dioctyl sulfonate and neostigmine.

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Congenital and Hereditary Conditions of Camelids

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The appearance of a congenital defect in any wild animal is cause for concern to keepers, zoo managers, and zoo veterinarians. Historically, congenital defects have been reported ever since animals were first maintained in captivity. Inbreeding is a constant hazard that may contribute significantly to increasing homozygosity in a small population of animals. The development of *species survival plans* (SSPs) in the past decade has made it possible to evaluate and scientifically share genetic lines to minimize the effects of inbreeding and linebreeding. However, challenges still exist.

Camelidae (camels, llamas, alpacas, guanacos, and vicuñas) were kept primarily as zoo animals in the United States until the 1970s, when the public became enamored of these animals and started to breed them privately as an alternative livestock enterprise. This chapter discusses the congenital/hereditary conditions of the camelidae.

Solving congenital defect problems begins with recognizing that many causes exist for congenital defects and are not necessarily hereditary (Box 48-1). Many factors may influence the well-being of the fetus, and numerous agents other than genetic factors may disrupt organogenesis.

TERMINOLOGY

Congenital condition Disorder present at birth. Unfortunately, some congenital defects may not be readily apparent at birth. For example, in humans and livestock, certain biochemical defects may not be visible until later in life. Conformation characteristics may not become apparent until after the cria has grown. In these cases, however, the foundation for the characteristic is present at birth.

Hereditary condition Disorder genetically transmitted from parent to offspring. The manifestation

of the condition may be present at birth or may develop subsequently.

Genetic disorder The term *genetic* is frequently used interchangeably with *hereditary*, but these are not synonymous. Certain genetic disorders may cause serious defects in a single individual, but the disorder will not be passed on to subsequent generations. However, reproduction may be impossible because of the nature of the defects.

Teratology The science of teratology (Greek *teras*, “monster”) deals with overall birth defects. A *teratogen* is any agent that causes abnormal development of the fetus. The furor over inadequate testing of drugs that may be prescribed for pregnant women was spawned by the thalidomide disaster of the 1970s. Both physical and chemical effects on the fetus were known before that time, but now an entire discipline of medicine and biology deals with such topics.

Teratogenesis Process by which teratogens exert their effect.

Genotype Genetic constitution of an individual at one or more loci.

Phenotype Observable trait of an individual.

Inbreeding Breeding of related individuals.

Inbreeding depression Decrease in performance resulting from inbreeding.

Linebreeding When an ancestor appears multiple times in a pedigree; typically used to foster a desirable trait from a superior ancestor.

Camelid All species of the camelidae.

SACs South American camelids (llama, alpaca, guanaco, vicuña).

CAMELID GENETICS

All camelids have a diploid chromosome number of 74. There are three pairs of *submetacentric* autosomes

Box 48-1**Potential Etiologies of Congenital Defects****Genetic**

Gene mutation
 Familial characteristics
 Chromosomal aberrations

Infectious Agents Damaging the Fetus**Physical Effects**

Trauma
 Hyperthermia
 Irradiation

Chemical

Drugs
 Poisonous plants
 Malnutrition
 Excesses
 Deficiencies

and 33 pairs of *acrocentric* autosomal chromosomes in camelids. The X chromosome is the largest submetacentric chromosome, and the Y chromosome is a very small acrocentric chromosome. Some confusion over the classification of camelid chromosomes has arisen among investigators, perhaps because of variations in staining procedures and evaluation at different phases of meiosis.⁶ Chromosome banding patterns and nucleolus organizer regions have also been identified, but a complete discussion of karyology is beyond the scope of this chapter. Fertile hybrids have been produced among all four species of SACs.

TERATOGENESIS

The causes of congenital and hereditary defects are manifold (see Box 48-1). Although no specific congenital defects caused by infectious agents have been reported in camelids, it seems likely that these may occur.

A number of viral infections are also teratogenic in humans, cattle, sheep, goats, swine, cats, and ferrets. The ultimate effects on the fetus are determined by the species involved, the strain of the virus, and the stage of pregnancy at the time of exposure to the teratogen.

Bovine virus diarrhea virus (BVDV) has caused cerebellar dysplasia, ocular defects, inferior brachygnathia, alopecia, internal hydrocephalus, and impaired immunologic competence in calves and lambs.⁴⁰

Bluetongue virus (BTV) has been shown experimentally to cause central nervous system (CNS) defects (hydrocephalus, cerebral hypoplasia, dysplastic spinal cord), retinal dysplasia, and arthrogryposis in lambs. Exposure of pregnant heifers to BTV resulted in abortion, arthrogryposis, prognathia, and a “dummy-calf” syndrome.⁴⁰ It is important to note that modified live virus (MLV) BTV vaccines may also exert teratogenic effects on the fetus of the pregnant ewe. The use of any MLV vaccine in any species other than those for which the vaccine was prepared is hazardous.

Both hog cholera virus and swine influenza virus are teratogenic. *Feline panleukopenia virus* (FPLV) causes cerebellar hypoplasia in kittens and ferrets.⁴⁰ Interestingly, mature ferrets are refractory to overt infection with FPLV, yet teratogenesis occurs. In humans, examples of teratogenesis include congenital syphilitic blindness and congenital deafness from prenatal infection with German measles virus.

Chemically induced teratogenesis is being intensively studied in humans, livestock, and laboratory animals. No chemical teratogenic defects have been identified in camelids. However, such effects are known to occur in all other species studied, so it should be expected that chemical teratogenesis will ultimately be identified in camelids. Some congenital defects already identified in camelids are induced by chemical teratogens in other livestock species. It should be pointed out that these defects are also known to be inherited traits in one or more species (Table 48-1). Veterinarians should investigate both possibilities when congenital deformities occur.

The following general principles should be understood:

1. The degree of susceptibility to the effects of a teratogen is determined by the genotype of the animal. Not all species are equally affected.
2. To affect the fetus, the teratogen must pass through the placenta in the metabolically active form.
3. The nature of the deformity is dose dependent. High doses of a certain teratogen at a critical time result in resorption. Slightly lower levels result in dead, deformed fetuses; still lower levels in living, deformed fetuses; and at the lowest levels, in normal, live offspring.
4. The fetus must be exposed to the teratogen at a specific period during gestation. Knowledge of embryology, especially the time and sequence of organogenesis, is fundamental to understanding teratogenesis.
5. Chemically dissimilar teratogens may produce identical effects on the fetus.

Table 48-1

Congenital Conditions of Camelids and Their Inheritability in Other Domestic Animals

Condition	Human ^{11,43}	Bovine ⁴²	Equine ²⁷	Ovine ^{10,51}	Caprine ⁴⁰	Porcine ²⁸	Canine ¹³	Feline ⁵²
Skeletal								
Ankylosis, carpus			U					
Angular limb deformity								
Carpal valgus	Y		S	S				
Carpal varus				U				
Femorotibial valgus								
Metacarpophalangeal valgus								
Arthrogryposis	Y	Y	Y	Y		Y		
Femur, shortened								Y
Hemivertebra		S					U	Y
Spinal agenesis								
Metacarpal shortening							U	Y
Patellar luxation	Y						Y	
Polydactyly	Y	Y	U	Y	Y	S	Y	Y
Scoliosis	Y	U		U				
Syndactyly	Y	Y		U				
Tail, agenesis				U		U	U	Y
Talus, vertical	Y							
Tendon contracture								
Carpus	Y			U				
Stifle				U				
Head/face								
Cerebellar hypoplasia	Y	Y	S	S		U	S	Y
Choanal atresia	Y							
Cyclopia	U	U	U	U	U	U		U
Encephalomeningocele	U			U				U
Facial bones								
Agenesis								
Lateral deviation			U					
Hydrocephalus, int. ⁶	Y		U	U		S	Y	Y
Mandible								
Brachygnathia		Y	Y	Y	Y	U	Y	
Micrognathia				Y		U		
Prognathia	Y		Y				Y	Y
Maxilla								
Brachygnathia						U	Y	
Prognathia	Y		Y				Y	
Nares, agenesis								
Nasal passage, stenosis							U	
Palate, agenesis								
Palatoschisis, cleft	Y	Y	U	Y		U		Y
Teeth, retention of deciduous								
Reproductive system								
Cervix, agenesis								
Cervix, double		Y						

Y, Inheritance confirmed; S, inheritance suspected; U, occurs, but etiology unknown; [blank], no information.

Continued

Table 48-1—cont'd

Congenital Conditions of Camelids and Their Inheritability in Other Domestic Animals

Condition	Human ^{11,43}	Bovine ⁴²	Equine ²⁷	Ovine ^{10,51}	Caprine ⁴⁰	Porcine ²⁸	Canine ¹³	Feline ⁵²
Reproductive system—cont'd								
Oviduct, segmental agenesis	Y	Y				U		
Hymen, imperforate	Y	S				U		
Intersex	U	U	Y	Y	Y	Y	U	U
Ovary, agenesis	Y	Y						U
Ovary, hypoplasia								U
Penis								
Corkscrew	U	U						
Curvature								
Hypoplasia	U	U	U					
Persistent frenulum		Y				U		
Testicles								
Cryptorchidism	Y	Y	Y	Y	Y	Y	Y	U
Cystic structures								
Ectopic		Y						U
Hypoplasia	Y	Y		Y				U
Twinning	Y	Y	Y	Y	Y			
Uterus								
Segmental agenesis		Y				U		U
Unicornus								U
Vagina, segmental agenesis	Y	Y		U				
Digestive system								
Atresia ani	Y	Y	U	Y		Y		
Atresia coli	Y	U	U					
Megaesophagus			U				S	S
Pyloric stenosis								
Cardiovascular system								
Atrial septal defect	U		U			U	U	U
Aortic arch, persistent, right ³⁶			U				Y	U
Ductus arteriosus, patent	Y		U			U	Y	S
Postcaval shunt								
Tetralogy of Fallot	U	U	U				Y	U
Transposition of great vessels			U			U		U
Ventricular septal defect	U	Y	U	Y		U	Y	U
Eye								
Blindness, cause not determined							Y	
Cataract	Y	Y	Y				Y	Y
Ectropion			U				U	
Entropion	Y	Y	U	Y		U	U	U
Eyelid, hypogenesis	Y							U
Iris, nonpigmented		Y	U			Y	Y	U

Table 48-1—cont'd

Congenital Conditions of Camelids and Their Inheritability in Other Domestic Animals

Condition	Human ^{11,43}	Bovine ⁴²	Equine ²⁷	Ovine ^{10,51}	Caprine ⁴⁰	Porcine ²⁸	Canine ¹³	Feline ⁵²
Miscellaneous								
Dwarfism	Y	Y	Y	Y	Y	Y	U	
Ear defects					Y	S		
Hernia								
Diaphragmatic*	Y		U			S	Y	Y
Inguinal		U	U			Y	U	U
Umbilical†	U	Y	Y	Y		Y	Y	Y
Renal agenesis	Y	U	U	U	U	U	S	U
Polythelia	Y	Y	Y	Y	Y	Y	U	U
Teat agenesis								
Toenails, crooked		Y	Y	Y		Y		
Patent urachus		U		U		U		U

*Ingram, Personal communication, 1983.

†Fowler, 1998.

Box 48-2 lists factors necessary for teratogenesis to occur.

Poisonous plant ingestion by a pregnant SAC is an ever-present hazard to the fetus.^{31,32} SACs are fastidious in their eating habits, rarely consuming large amounts of strange plants, but they do investigate and try new plants. A low-dose intake may be a saving factor in camelids. Table 48-2 lists plants known to produce teratogenic defects in livestock.

Perhaps of even greater importance are the potential effects of chemical agents on the reproductive process without obvious outward expression. Teratogens may have a direct effect on ova or spermatozoa, causing infertility. High doses early in gestation may cause fetal death with resorption or undetected abortion. Lethal effects may be the result of maternal ingestion early in gestation, even though fetal death occurs late in gestation or postpartum. Nonlethal effects may either prevent reproduction or allow reproduction of

constitutionally unsound individuals that may be highly susceptible to other diseases.

HEREDITARY TRAITS

Hundreds of anatomic and physiologic traits are passed from parents to offspring by gene pairing.^{38,44} Genome research and gene mapping have progressed at astronomical rates, but to my knowledge, a gene map of a camelid has not been produced.

Fiber coat color inheritance in llamas and alpacas has received attention by both South American²² and North American^{24,33,34,41} investigators, but definitive genetic studies in camelids have not been conducted and reported.

Body conformation is inherited, but not in a typical mendelian fashion with simple dominant and recessive genes. Numerous genes may influence conformation positively or negatively. Environment and nutrition are also factors.

Box 48-2

Factors Necessary for Teratogenesis

1. Causal agent is frequently species specific.
2. Organ affected may be specific.
3. Toxin must pass through the placenta.
4. The effect is dose dependent.
5. Time of exposure during gestation is critical.
6. Multiple toxins may produce the same defect.

CHROMOSOMAL ABERRATIONS

A number of chromosomal abnormalities have been reported in domestic animals, but not in camelids. These defects occur during meiosis and include fusion of chromosomes, translocations of segments of chromosomes, loss of a segment, and other changes.²⁶ The expression of the defect is determined by whether or

Table 48-2**Plants Known to Be Teratogenic**

Genus/Species	Common Name	Animals Affected
<i>Astragalus</i> spp.	Locoweed	Cattle and sheep
<i>Conium maculatum</i>	Poison hemlock	Cattle
<i>Datura stramonium</i>	Jimsonweed	Pigs
<i>Lupinus</i> spp.	Lupine	Cattle
<i>Nicotiana tobaccum</i>	Tobacco	Pigs
<i>Sorghum vulgare</i>	Sudan grass	Horses
<i>Veratrum californicum</i>	False hellebore, corn lily	Sheep

not the change occurred on an autosomal pair or one of the sex chromosomes. Chromosomal aberrations may affect a single individual or may be perpetuated as inherited characteristics. Chromosomal aberrations may be identified by a combination of family pedigree analysis, identification of interspecific somatic cell hybrids, and cytogenetic studies, including karyotyping and various banding staining.

Genetic studies have been developed to the stage of highly technical submolecular, biochemical, and deoxyribonucleic acid (DNA) complexities. Camelid inheritance is still in the descriptive stage. Although most of the congenital defects reported in camelids are known to be inherited in one or more species of other domestic animals or humans, it is important to recognize that many may also be produced by other etiologic agents (see Box 48-1). In fact, such defects as arthrogryposis are more likely to be caused by exposure of the dam to a toxic substance at a crucial time during gestation than by genetic damage.

DETECTION OF INHERITED TRAITS

Circumstantial evidence that a congenital trait may be hereditary is based on evidence as follows^{6,46}:

1. That the trait is known to be inherited in two or more other species.
2. That the trait appears multiple times in a pedigree chart; this may require statistical analysis.
3. That the trait appears with greater frequency in a species, breed, or population.
4. The use of breeding trials. This has been used successfully in cattle when trying to prove the freedom of a carrier genetic trait of a bull used in an artificial insemination stud.
5. Laboratory analysis for certain biochemical anomalies.

The detection of an inherited trait depends on the mode of inheritance. If a characteristic is dominant, one of the parents will be phenotypically positive and, generally, at least 50% of its offspring will express the phenotype. However, even though a characteristic may be dominant, environmental or genetic factors may affect the degree of expression of a phenotype.

The majority of inherited defects are recessive, and both parents must contribute the gene in order for the offspring to exhibit the trait. Recessive traits may be *simple*, in which only one gene is involved, or *multifactorial*, which complicates expression and detection in a population.

The diagnosis of an inherited trait is a laborious, costly, time-consuming process. Familial repetition is the most important data necessary, and this requires detailed genealogy of both normal and abnormal offspring. The ultimate evaluation is based on breeding trials. Only one major camelid breeding trial is currently under way, to establish the etiology of choanal atresia in llamas and alpacas.

BREEDING MANAGEMENT SYSTEMS

It is beyond the scope of this chapter to discuss all the various breeding management systems. Because some of these systems have profound influence on the prevalence of congenital defects, veterinarians should consult contemporary books on the subject.⁴⁶

Inbreeding is a mating system in which the progeny produced by parents are more closely related than the average of the population from which they come. Father-daughter, brother-sister, grandfather-granddaughter, and other close-relationship breedings have been carried out with llamas, both intentionally and unknowingly. Often, the parentage of some llamas is unknown, and only recently has it become possible to verify parentage

in llamas and alpacas, as routinely done in cattle and horses.^{47,48}

Inbreeding increases homozygosity and is used in livestock breeding to strengthen a given characteristic. Unfortunately, it may also concentrate undesirable traits. Inbreeding is a technique that should be practiced only by highly skilled and experienced breeders who are willing to cull (not sell) individuals exhibiting undesirable traits.

In general, inbreeding is followed by a decline in traits closely related to physical fitness, such as fertility, mothering ability, viability, and growth rate.⁴⁹ Detailed records were kept on a herd of inbred dorcas gazelles (*Antidorcas gazellei*) at a zoo. As the inbreeding coefficient increased, so did the neonatal mortality rate. The calves died of inanition, weakness, white muscle disease, and a variety of other infectious and noninfectious diseases. The neonates lost their "coping" ability.⁴⁹ Veterinarians should be fully cognizant of the ramifications of the practice of inbreeding.²⁵

Linebreeding is a form of inbreeding in which an attempt is made to concentrate the inheritance of some one ancestor or ancestral line in a herd.

CONGENITAL CONDITIONS

Table 48-1 lists congenital conditions that have been identified by me,¹⁵⁻¹⁹ reported in the literature,* or reported by personal communications. The inheritability of similar conditions in humans and other animal species is indicated, if known. Boxes 48-3 and 48-4 list and compare congenital conditions in camelids and other domestic animals. The reader is again reminded that these conditions may have other than hereditary causes. Some of the conditions are discussed in detail because of their prevalence; others are only listed.

SKELETAL DEFECTS

Angular Limb Deformity

Conformation of the limbs in association with the body is an inherited trait in all animals. There appears to be a high prevalence of crooked legs in llamas and alpacas. Carpal valgus is most prevalent,¹⁵ but carpal varus, metacarpophalangeal valgus, and femorotibial valgus have also been seen.

Although there is evidence that some forms of angular limb deformity are familial,¹⁵ all types should

Box 48-3

Congenital Conditions Known to Be Inherited in Other Species (≥2)*

Arthrogryposis (5)
 Polypodia (polydactyly) (5)
 Syndactyly (3)
 Cerebellar hypoplasia (3)
 Internal hydrocephalus (3)
 Mandibular brachygnathia (5)
 Mandibular prognathia (3)
 Maxillary brachygnathia (3)
 Cleft palate (4)
 Segmental agenesis of oviducts (2)
 Vaginal segmental aplasia (2)
 Ovarian agenesis (2)
 Twinning (5)
 Intersex (4)
 Cryptorchidism (7)
 Hypoplastic testes (3)
 Atresia ani (4)
 Patent ductus arteriosus (2)
 Ventricular septal defect (3)
 Cataract (5)
 Entropion (3)
 Nonpigmented iris (glass eye) (3)
 Dwarfism (6)
 Hernia
 Umbilical (7)
 Diaphragmatic (3)
 Polythelia (multiple teats) (4)
 Crooked toenails (4)

*Should be considered the same in camelids.

not be placed in the same etiologic and diagnostic category (Figure 48-1). Nutrition is thought to be a factor in some cases, trauma in others, whereas in many cases the true cause is unknown. This is also true in the horse. Angular limb deformity is a common sequela to rickets in crias. The cause of the bowing is different (cortical thinning) in rickets, as is the radiographic picture.

In *carpal valgus* the prominent sign is inward bowing of one or both carpi. If the defect is allowed to progress, the carpi overlap each other when the animal is standing still. Abrasions at the medial aspect of the carpi may be noted from the trauma of the carpi knocking against one another when walking. In one individual the deviation was so severe that the legs were actually crossed, although when the animal was viewed from the front, the legs appeared to be straight because hair hid the crossed upper forearm and arm. Outward bowing of the carpi is rarer.

Carpal valgus may be present at birth, but an evaluation should be delayed for a month to allow normal

*References 1, 2, 4, 8, 9, 21, 25, 30, 41, 53, 59, 60.

Box 48-4**Congenital Defects Highly Suspected of Being Hereditary**

Choanal atresia
 Familial in humans
 High prevalence in the population
 Megaesophagus
 Ear pinna defects
 Wryface
 Renal agenesis
 Ovarian hypoplasia
 Horizontal talus
 Hemivertebrae
 Carpal valgus
 Metacarpophalangeal valgus
 Eyelid hypogenesis

straightening to occur. The degree of deviation may develop at 2 to 6 months of age and progressively become more severe, up to 15 months of age. However, even in cases of development a few months after birth, a spontaneous correction of the deviation may occur as the animal grows.

Radiographs should be taken to evaluate the carpus and contiguous long bones. The ulnar physis is approximately 3 to 5 cm (1-2 inches) proximal to the radial physis. The ulnar epiphysis extends distally along the lateral radius and attenuates as it becomes a part of the radial epiphysis. Radiographs and anatomic preparations show no physical separation of these two epiphyses in the majority of individuals. However, I have examined some animals with separated epiphyses.

Variable radiographic changes may be observed in the carpal region. Lesions of metabolic bone disease are rarely observed. Inflammation of the radial physis, characterized by flaring and widening of the physis, may be caused by trauma. The typical radiographic lesion of carpal valgus is a wedge-shaped radial epiphysis, with the base of the wedge on the medial aspect of the carpus. The width of the radial physis is variable, but the ulnar physis is flared, doubly cupped in shape, with hyperplasia of the distal ulna.

The pathogenesis of carpal valgus appears to be a cessation of growth at the ulnar physis on the lateral aspect of the limb, allowing continued growth of the medial radial physis, producing inward bowing of the limb at the carpus.

Splints (polyvinyl chloride pipe cut lengthwise) may be applied to the limbs of young animals (<2 months of age) with mild deviation. It is unlikely



Fig 48-1 Angular limb deformity in llama. (See Color Plate 48-1.) (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)

that any appreciable straightening of the limb will occur after the animal is 15 months of age, although the physis may not be entirely closed until 3 years of age.

Patellar Luxation

I have dealt with two cases of congenital bilateral medial luxation of the patella in llamas (Figure 48-2). Others have also observed lateral luxation. Full medial luxation causes the cria to stand in a crouched position. The stifle joint is thickened, and the patella is palpated in the medial position rather than in the dorsal groove of the femur. It is impossible to manipulate the patella to the normal position.

Upward fixation of the patella may be an acquired condition in older llamas. A predisposition may be a congenital conformational weakness (straight rear limbs, laxity of tendons and ligaments). The mechanism for upward fixation in a camelid is different than in a horse. The distal patellar ligament is a sheet of tendinous tissue rather than one, two, or three discrete ligaments. With laxity of the tibiopatellar and femoropatellar ligaments, the patella may lodge at the dorsal tip of either the medial or the lateral ridge of the trochlea.

The prognosis for medial patellar luxation is unfavorable.



Fig 48-2 Medial patellar luxation in llama cria. (See Color Plate 48-2.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)

Polydactyly and Syndactyly

Polydactylism (*polydactyly*) is a common congenital defect in llamas and alpacas⁵¹; one to three accessory digits may occur on one or all four limbs. Fusion of two normal digits (*syndactyly*) is less common. Both conditions have been identified as inherited traits in cattle, dogs, and humans. The conditions are evident on clinical examination. The degree of development of the accessory digits varies, but it may be complete, with a full complement of tendons, ligaments, and bones, including metacarpals and metatarsals. Polydactyly is frequently seen in multiple-anomaly situations. The genetics of this trait are not known for camelids; however, Figures 48-3 and 48-4 illustrate the trait in a dam and her fetus, suggesting heritability. Native pastoralists in Peru believe it to be good luck to have a polydactyl animal.

Craniofacial Defects

A number of congenital defects of the face, nasal cavity, and pharynx of llamas and alpacas may be lethal because of the obligate nasal breathing of the cria (see Table 48-1). The precise relationship between the various defects is unknown. The embryologic development of the nasopharyngeal region is complex. The least evident defect may be stenosis of the nasal passages.

Choanal atresia is common and may consist of a membranous or osseous partition between the nasal and pharyngeal cavities^{14,26} (Figure 48-5). Agenesis of



Fig 48-3 Polydactylism in llama female. (See Color Plate 48-3.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Fig 48-4 Polydactylism in llama fetus from female in Figure 48-3. (See Color Plate 48-4.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)

the facial bones causes variable shortening of the face and muzzle and accentuates the doming of the forehead, which may be mistaken as a hydrocephalic condition. In extreme cases, agenesis of the facial bones, along with other tissue dysgenesis, may result in cyclopia.

The etiology of camelid facial dysgenesis is unknown. Teratogens are known to produce similar congenital defects in sheep. A familial relationship is known in humans with choanal atresia.¹² Clinical signs vary with the nature of the defect; all produce some impairment of respiration. Complete occlusion of the nasal passageways causes a characteristic breathing pattern in the cria. On inspiration, the mouth is opened slightly and filled with air. The lips are then slightly closed while air continues to be sucked into the mouth, ballooning the cheeks. The lips close tightly, and the



Fig 48-5 Choanal atresia in llama neonate. (See Color Plate 48-5.) (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)

cheeks compress to force air around the elongated soft palate, which is positioned ventral to the epiglottal cartilage. With expiration, air is forced out of the larynx into the oropharynx, and complete exhalation requires further effort to push the air around the soft palate and out the mouth.

An affected cria stands with the head extended because this position restricts air flow the least. Also, the extended-head position may allow the soft palate to be flipped dorsal to the epiglottal cartilage, permitting free flow of air into the trachea. During this time, breathing is more normal.

Restricted expiratory airflow entraps excessive air in the pharynx, which in turn may be swallowed, causing tympanites. Affected crias not only have difficulty breathing but also find it almost impossible to nurse. The time required to obtain sufficient oxygen to sustain life precludes time for swallowing milk. Aspiration pneumonia is a common sequela. Affected crias are known to chew at and ingest fiber from the mother. Numerous hair balls have been observed in compartment one (C-1) of the stomach at necropsy.

Nasopharyngeal obstruction may be partial or complete and unilateral or bilateral. Some affected animals are not detected as neonates but have respiratory deficiencies as an adult. Such conditions are difficult to differentiate from acquired chronic respiratory diseases.

Definitive diagnosis of these conditions may require radiographs. In a neonate with suspected choanal atresia, a 5-mm catheter should be inserted intranasally. The muzzle should be maintained in an elevated position to prevent backflow of the medium from the nostril; 10 mL of a contrast medium (e.g., Hypaque) should be deposited into the nasal cavity, followed by immediate exposure of both a lateral and a dorsoventral view. Both nasal cavities should be evaluated.

The prognosis for the life of a cria with choanal atresia is unfavorable.



Fig 48-6 Superior brachygnathism in alpaca. (See Color Plate 48-6.) (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)



Fig 48-7 Retained deciduous incisors in llama. (See Color Plate 48-7.) (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)

Jaw Dysgenesis

The most common congenital disorders of llamas and alpacas involve malformations of the mandible or maxilla (Figure 48-6). There is little question that the various forms of shortening or elongation of the jaws are hereditary. Genetic transmission is known in other livestock, pets, and humans (see Table 48-1).

Although alpacas have continuously growing incisors, with proper alignment the teeth are naturally worn off and overgrowth does not occur. Retention of deciduous incisors is common, and a familial tendency is suspected in SACs, but the precise etiology is unknown (Figure 48-7).

Reproductive System Defects

Much has been written about congenital and hereditary defects of the reproductive system of llamas and alpacas. Many of the defects seriously impair or prevent reproductive performance. The prevalence of reproductive defects in both North America and South America is alarming. In one study in Peru, as many as 10% of the animals had one or more defects.^{59,60} Veterinarians should be aware of the scope of the problem and should consider these conditions on any soundness or infertility examination.

Hypogenesis or Agenesis of Reproductive Organs

Failure of development, particularly of female reproductive organs, is often seen. The etiology is unknown in camelids, but genetic transmission has been reported in cattle and humans (see Table 48-1). The major clinical sign is infertility, although pregnancy is possible in some cases, such as animals with uterus unicornis.

Segmental agenesis of the tubular genital tract of the female is common. Stenosis or occlusion of the tract may occur at any location from the oviduct to the hymen. Unilateral agenesis of an oviduct may never be detected. Agenesis within the uterus or vagina prevents outflow of uterine secretions, resulting in accumulation of fluid (*mucometria*) and dilation of the segments of the tract cranial to the agenesis (Figure 48-8).

The dilated uterus may be mistaken for pregnancy on rectal palpation. The fluid may be milky to slightly reddish in color and may have the consistency of skim

milk. The fluid is sterile unless organisms have been introduced by diagnostic manipulation. It is difficult to differentiate mucometria from placental fluids on palpation, but it is easy to differentiate it from pyometra on ultrasonography. Exudates caused by pyometra have a flocculent appearance, whereas mucometria and placental fluids are homogeneously clear.

The diagnosis of segmental agenesis involves a combination of rectal palpation, ultrasonography, examination with a vaginal speculum, aspiration of fluid, and visualization at laparotomy or laparoscopy, depending on the location of the lesion. Ovarian hypogenesis and agenesis also occur.

Male Defects

Any of a long list of hereditary defects of male livestock species warrants exclusion of the individual from breeding.²⁶ Such congenital defects of male camelids include testicular hypoplasia, cryptorchidism, testicular cysts, penile hypoplasia, persistence of the penile frenulum, and curvature of the penis. It should be recommended to clients that such defects warrant exclusion of males for breeding.

Digestive Tract Defects

Atresia ani and atresia coli are known to occur in camelids⁹; the etiology is unknown. In cattle, atresia coli has been associated with excessive pressure exerted during rectal palpation.⁴² Megaesophagus may be either congenital or acquired; again, the etiology is unknown.



Fig 48-8 Mucometria caused by segmental agenesis of llama vagina. (See Color Plate 48-8.) (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)



Fig 48-9 Ventricular septal defect in llama. (From Fowler ME: *Medicine and surgery of South American camelids*, ed 2, Ames, Iowa, 1998, Blackwell.)

Cardiovascular Defects

Cardiovascular defects are seen sporadically in most animal species; none is unique to camelids (see Table 48-1).

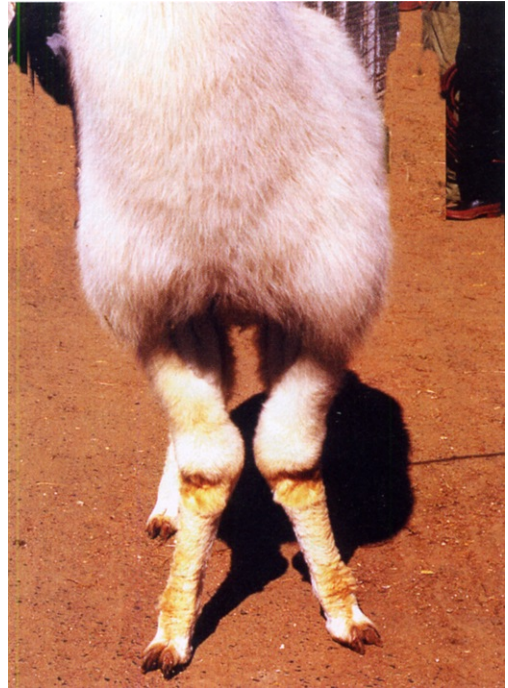
Ventricular septal defect (VSD) is relatively common in llamas and may occur alone or may be associated with other cardiovascular or congenital defects (Figure 48-9). Clinical signs may be limited to auscultation of a holosystolic murmur or may be accompanied by cyanosis and exercise intolerance. I am aware of an adult llama with VSD that lived a normal life. The presence of a murmur is a frequent, perhaps normal, clinical finding in the newborn. The murmur should disappear by 1 week of age.

The diagnosis of VSD should be suspected with the presence of a murmur but may be definitively diagnosed with ultrasonography. Other cardiovascular defects may be diagnosed only through radiography, fluoroscopy, or angiography.

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Color Plate 48-1 Angular limb deformity in llama. (For text mention, see Chapter 48, p. 398.) (*From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.*)



Color Plate 48-2 Medial patellar luxation in llama cria. (For text mention, see Chapter 48, p. 399.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-3 Polydactylism in llama female. (For text mention, see Chapter 48, p. 399.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-4 Polydactylism in llama fetus from female in Figure 48-3. (For text mention, see Chapter 48, p. 399.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



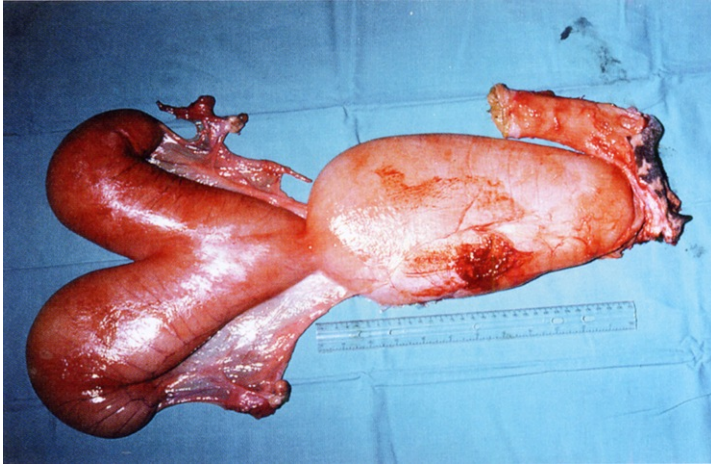
Color Plate 48-5 Choanal atresia in llama neonate. (For text mention, see Chapter 48, p. 400.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-6 Superior brachygnathism in alpaca. (For text mention, see Chapter 48, p. 400.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-7 Retained deciduous incisors in llama. (For text mention, see Chapter 48, p. 400.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-8 Mucometria cause by segmental agenesis of llama vagina. (For text mention, see Chapter 48, p. 401.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)



Color Plate 48-9 Ventricular septal defect in llama. (For text mention, see Chapter 48, p. 402.) (From Fowler ME: Medicine and surgery of South American camelids, ed 2, Ames, Iowa, 1998, Blackwell.)

Hypocalcemia, Hypomagnesemia, and Rumenitis in Exotic Ruminants

MICHELE A. MILLER AND MARTHA WEBER

As nutritional products provided to exotic ruminants have improved in quality to simulate those for domestic ruminants, an associated increase in the prevalence of subclinical and chronic disease states may be attributable to high-quality feeds and the feeding management of these species. An increased prevalence of hypomagnesemia and hypocalcemia, both subclinical and clinically associated with tetany, has been observed in multiple collections of exotic ruminants. Other manifestations of disease associated with systemic imbalances of calcium (Ca^{++}) and magnesium (Mg^{++}) are chronic laminitis and decreases in food consumption and body condition. Zoo practitioners have often observed inverse calcium/phosphorus (Ca/P) ratios in serum chemistry panels from captive ungulates receiving apparently balanced diets.

Because Mg^{++} is often not included in routine analysis, hypomagnesemia may be unrecognized. Persistent hypocalcemia paired with a sudden drop in serum Mg^{++} associated with fasting or decreased feed intake may lead to acute tetany. Chronic manifestations of hypocalcemia and hypomagnesemia, such as lameness and chronic laminitis, are often attributed to other causes, such as poor substrate or inadequate foot care.

The species in which this syndrome has been most often recognized are nyala (*Tragelaphus angasii*), kudu (*Tragelaphus imberbis* and *T. strepsiceros*), and eland (*Taurotragus oryx*), with bongo (*Tragelaphus eurycerus*) and giraffe (*Giraffa camelopardalis*) less frequently exhibiting acute manifestations. However, any ruminant species may be considered at risk.

REVIEW OF MINERAL METABOLISM

Role of Magnesium

From 60% to 70% of the body's magnesium is found in bone and is not available to maintain serum

Mg^{++} levels in adult animals.³ Magnesium is the second most common intracellular cation after potassium.⁶ Because the pool of available Mg^{++} is small, magnesium must be continuously ingested in the diet to maintain adequate systemic levels. There is minimal hormonal control over Mg^{++} homeostasis, and serum levels vary with dietary intake and renal excretion.¹⁴

Magnesium plays a role in muscle contraction, energy metabolism, and Ca^{++} metabolism. It is an essential mineral for enzyme activation, especially those reactions that use adenosine triphosphate (ATP).¹⁴ It is also important in the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and proteins and as a modulator of synaptic transmission in skeletal muscles. Low Mg^{++} levels potentiate the release of acetylcholine (ACh) at neuromuscular junctions and may lead to tetany caused by increased ACh concentrations at motor end plates.¹⁴ Changes in ACh concentrations may also induce muscle weakness, cardiac arrhythmias, and ileus. Systemic inflammatory responses have been reported to be of increased severity in patients with subclinical hypomagnesemia.¹⁵

Typically a disease of pastured cattle, acute signs of hypomagnesemia are often seen after ruminants are put on lush pasture ("grass staggers") and may manifest as severe tetany. Chronic hypomagnesemia may manifest as anorexia, abnormal gait, poor growth rate or body condition, hyperexcitability, twitching ears, kicking at the abdomen, bruxism, hypersalivation, tetany, seizures, and unexpected death. The development of clinical signs associated with hypomagnesemia is often associated with lactation, stress, transport, or anorexia.^{6,10} Stress may exacerbate previously subclinical hypomagnesemia because sympathetic nervous system activation causes epinephrine release, which results in decreased plasma Mg^{++} .⁶

Role of Calcium

The majority of the body's calcium is stored in the skeleton, with only 1% found in intracellular and extracellular fluids.¹¹ Extracellular Ca^{++} plays a significant role in nerve and muscle function as well as numerous enzymatic processes. Hypocalcemia may be a result of a dietary deficiency or imbalance or a disruption of normal Ca^{++} homeostasis within the animal. Systemic Ca^{++} balance depends on interactions among parathyroid hormone (PTH), vitamin D, and calcitonin. PTH and vitamin D promote increases in serum Ca^{++} , whereas calcitonin causes decreased intestinal absorption and increased renal excretion of Ca^{++} . Hypomagnesemia in cattle may lead to decreased PTH secretion as well as decreased tissue responsiveness to PTH, resulting in impaired absorption and retention of Ca^{++} .⁸

Acute signs of hypocalcemia may include muscle stiffness or tetany, decreased rumen motility, and death. Evidence of a chronic Ca^{++} deficiency may manifest as poor feed intake, slow growth, rickets or osteomalacia, and pathologic fractures.

Dietary Sources of Calcium and Magnesium

Legumes generally are an excellent source of dietary calcium, whereas grains have lower concentrations of available calcium. Absorption of Ca^{++} from the diet is affected by multiple factors, including the presence of oxalates, concentrations of Ca^{++} and phosphorus in the feed, and relative balances of PTH, calcitonin, and vitamin D. Dietary Ca^{++} uptake is hormonally regulated in the small intestine.

Grains have higher levels of available Mg^{++} than most forages¹²; legumes also have relatively high Mg^{++} concentrations.³ Magnesium is both actively and passively absorbed in the rumenoreticulum in adult ruminants and the small intestine in preruminant calves.¹⁷ Interference with Mg^{++} absorption from the forestomach may lead to rapid development of hypomagnesemia both because substantial amounts of Mg^{++} are secreted into the saliva of ruminants³ and because body stores of Mg^{++} are not easily mobilized in times of deficiency. High forage potassium (K^+) levels, such as those found in lush grasses, will interfere with rumen uptake of Mg^{++} by changing electrochemical gradients across the rumen epithelium.⁵ Other causes of impaired Mg^{++} absorption include sudden increases in rumen ammonia, high dietary Ca^{++} levels, and low dietary sodium (Na^+) levels. Low Na^+ results in an aldosterone-induced increase in K^+ secretion into saliva and the rumen.⁷

The resulting high rumen K^+ concentration results in decreased Mg^{++} absorption.

RUMEN ACIDOSIS AND RUMENITIS

Rumen acidosis may be one of the underlying causes of chronic mineral imbalances in exotic ungulates. Decreased rumen pH is associated with intake of highly fermentable carbohydrates, decreased effective fiber intake, stress, and overall decreased feed intake. Any of these factors may lead to a shift in rumen microflora and may change normal fermentation patterns toward acid production. Ungulates with acute acidosis may have loss of normal rumen flora, chemical damage to the rumen epithelium, and development of systemic acidosis. This may result in disruption of nutrient absorption in the rumen and microbial invasion of damaged rumen tissues, leading to bacterial or mycotic rumenitis and possible endotoxin release. One hypothesis for the development of laminitis in animals with rumen acidosis and rumenitis is the release of histamine, endotoxins, and septic emboli during the disease process.⁴

In cattle, chronic rumen acidosis appears to lead to chronic rumenitis. The inflammatory changes include both ulceration and focal abscess formation in the rumen wall and have been associated with liver abscess formation and chronic laminitis. Laminitis may develop within 45 hours after cattle are fed a high-carbohydrate ration, which may occur in the absence of rumen lesions.¹⁶ In many cases the affected animals may not show any clinical signs related to acute disease.

Changes in rumen pH may also be related to feeding practices. Feeding large quantities of concentrates at one time, as may occur in some management systems, decreases forestomach pH. Inappropriate fiber particle size and quality of roughage in the diet also contribute to chronic rumen acidosis and rumenitis. Increased fiber and particle size require more rumination and chewing, leading to an addition of bicarbonate from the saliva as well as recycling of minerals.²

CLINICAL SYNDROME IN EXOTIC UNGULATES

Acute clinical signs caused by hypomagnesemia and hypocalcemia have been most often seen in captive exotic ruminants after immobilization. Animals may have difficulty rising after reversal, remain recumbent while appearing abnormally calm, or start to stagger

within a few hours of reversal. Clinical signs may be mistaken for renarcotization. Muscle fasciculations or “shivers” may precede a seizure or tetany.

It is suspected that these animals have subclinical rumenitis, with impaired mineral absorption leading to systemic mineral imbalances. The preimmobilization fasting period, along with additional stress associated with the anesthesia, results in serum Ca^{++} and Mg^{++} levels below the threshold for the manifestation of clinical disease. Often a review of prior serum chemistry panels for these individuals reveals chronic inverted Ca/P ratios and marginal Mg^{++} values, suggesting a chronic subclinical condition.⁹

Other clinical presentations include dystocia, retained placenta, and birth of weak calves.⁹ Animals with reproductive signs may also exhibit muscle fasciculations or evidence of chronic hoof disease.

Indirect evidence of chronic rumenitis and mineral deficiencies may include a history of intermittent lameness and signs of abnormal hoof growth with evidence of laminitis. Poor hair coat quality and body condition may also be observed. A unique presentation of laminitis has been observed in kudu hooves, in which a vertical crack on the medial aspect of the claws is often present, along with the more classic laminitic lines and overgrown toes.

Diagnosis

In acute cases a presumptive diagnosis may be made based on clinical signs, especially if tetany is present. In domestic cattle, hypomagnesemia is defined as serum Mg^{++} less than 1.2 mg/dL. A low cerebrospinal fluid (CSF) Mg^{++} level (<1.45 mg/dL) is also concurrently present but usually not measured.³ In the majority of cases, hypocalcemia with an inverse Ca/P ratio will also be present. Three male greater kudu (*Tragelaphus strepsiceros*) showing clinical signs had Ca^{++} values of 6.7, 4.5, and 6.8 mg/dL; phosphorus values of 14.6, 13.5, and 12.0 mg/dL; and Mg^{++} values of 1.0, 0.77, and 0.9 mg/dL, respectively.⁹

Affected nyala (*Tragelaphus angassi*) from another zoologic park provide evidence that this syndrome is present in more than one collection of exotic ruminants. This herd had been fed ad libitum alfalfa hay and commercial herbivore pellets (starch content >20%). Twenty of 21 animals were considered to be hypocalcemic (range, 6.2–8.1 mg/dL), with a mean serum Mg^{++} concentration of 0.95 mEq/L. When the herd was fed only browse and alfalfa hay for 5 weeks and retested, there was a significant increase in mean Ca^{++} and Mg^{++} (1.74 mEq/L) values for the herd.¹

Treatment

Treatment of acute clinical signs requires intravenous (IV) administration of calcium and magnesium. The dosages are based on those used to treat hypomagnesemia and hypocalcemia in cattle. Dextrose-containing fluids are not recommended during treatment because insulin will dramatically decrease serum Mg^{++} .¹⁸ Animals may have systemic acidosis, so the judicious use of bicarbonate may be indicated. Diazepam or other muscle relaxants may be used in an attempt to minimize secondary myopathy associated with fasciculations, as well as provide adjunctive therapy for seizures. Treatment of hypocalcemia alone is usually ineffective in reversing the clinical signs; magnesium must be administered as well. It is recommended that clinicians working with collections of exotic ruminants stock both injectable calcium gluconate and magnesium sulfate solutions. Rehydration and diuresis are critical to minimize the risk of myopathy and secondary renal complications. Once the animal is eating again, oral phosphate binders may be administered, along with rumen buffers that contain Ca^{++} and Mg^{++} until dietary adjustments may be made.

In those animals that appear to be less severely affected, subcutaneous administration of calcium gluconate and magnesium sulfate in separate pockets of lactated Ringer's solution appears to provide therapeutic benefit without adverse effects. Dosages are the same as for the IV route, but the solutions may be administered at a more rapid rate. Because of the volumes required, restraint and handling of the patient are usually necessary.

For chronically affected animals, commercially available cattle formulations that contain Ca^{++} , Mg^{++} , and buffers may provide mineral supplementation and may treatment of rumen acidosis. Often these products are labeled as “dairy buffers” or “therapeutic bloat products.”

Supportive care may also include analgesics for laminitis, antibiotics for possible bacterial overgrowth or septicemia, and low-dose flunixin meglumine (0.25 mg/kg once daily). Ungulates with signs of laminitis may benefit from transfer to an enclosure pen with a deep, soft substrate such as sand, which may provide support and prevent further damage. Aggressive management of laminitic hooves by frequent trimming may help to minimize P3 rotation, sole bruises, and abscess formation. Treatments used in laminitic horses, such as isoxsuprine and pentoxifylline, have been used in exotic ungulates anecdotally. Periodic radiographs of hooves affected by laminitis are recommended because of a preponderance of P3

fractures observed in these animals, especially kudu (Miller, personal observation).

Prevention

Addition of buffers, chelated minerals, and appropriate sources of fiber may ameliorate rumen acidosis and rumenitis. The current recommendation for captive herbivores is a pelleted diet that contains less than 3% starch. An experimental diet has been produced that eliminates grains and uses soy hulls, aspen, dried beet pulp, oat hulls, flax oil, and other ingredients to increase the acid detergent fiber from the traditional 16% to 32%. In addition, fermentable fibers such as pectins have been used to improve rumen fermentation, as have buffers and chelated minerals. The goal for using concentrates is to supplement forages so that the diet is balanced in nutrients. So little is known about the nutritional ecology and requirements of many of the species kept in zoologic collections that balancing diets based on recommendations for domesticated cattle may lead to multiple nutrition-related problems.

Since changing to this new diet and modifying feeding management practices, improvements in blood values in our (MM) ungulate collection have been observed, and no acute manifestations of hypomagnesemia and hypocalcemia have occurred.

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Diseases of Chamois

CHRISTIAN WALZER

BIOLOGY

The chamois is an artiodactylid of the Bovidae family, Caprinae subfamily. Two species of chamois are described: *Rupicapra pyrenaica* occurs in the Pyrenees and adjacent mountain ranges of northwestern Spain, and *Rupicapra rupicapra* is distributed throughout the Alps and the northern mountain ranges separating Poland and the Czech Republic and toward the east in Turkey, the Caucasus, and the Balkan region.³⁴ A subspecies (*Rupicapra p. ornata*) only occurs in the Abruzzo National Park in the Apennines mountains of Italy and is listed in Appendix 1 of the Convention on International Trade in Endangered Species (CITES).³ The southern chamois (*R. pyrenaica*) is listed at "lower risk" but "conservation dependent" by the International Union for Conservation of Nature and Natural Resources (IUCN, World Conservation Union).¹³

In the wild, *R. rupicapra* is widely distributed; current population estimates for Switzerland alone exceed 100,000 individuals.⁷ However, some local populations of chamois, such as the small Greek chamois population belonging to the Balkan subspecies (*R. r. balcanica*), are under pressure because of poaching and habitat degradation. Several other subspecies are listed as threatened by the IUCN: *R. r. caucasica* is classified as "vulnerable" and *R. r. tatrica* as "endangered," whereas *R. r. cartusiana* is classified as a "critically endangered" subspecies. Data are insufficient as to the current status of *R. r. asiatica*.¹³

At the end of the last Ice Age, the chamois retreated to the various mountain ranges. At present the species is naturally colonizing new biotopes, such as the Mediterranean region of Piermont in Italy. Furthermore, the chamois has been successfully reintroduced into the Swiss Jura and the Black Forest and Saxony in Germany, and it constitutes one of the many alien species of New Zealand.

The chamois is a prized hunting trophy throughout its distribution range. The meat is greatly valued, and the skin is made into "shammy" leather, which is widely used for polishing cars. Of particular tradi-

tional interest in southern Bavaria and Austria is the highly prized winter hair (1 g costs about 30 Euro) from the back of the chamois, which is bound into ornamental "Gamsbaerte" (see, e.g., www.gamsbart.at).

Chamois spend the warmer months of the year on higher-elevation alpine meadows. In the late fall and winter, the animals descend to lower elevations and at times move into forest areas. When disturbed, a sentinel animal stomps its foreleg and emits a high-pitched whistling alarm call. The animals flee at high speeds (50 kph, 30 mph) into the cliff faces. Chamois are particularly good climbers and may use the smallest of cracks to advance nimbly up a rock face. They are reported to jump at least 2 m (6½ ft) in height and 6 m (20 ft) in length.³²

There is a distinct seasonal variation in social structure in this species. In the spring the females leave the groups to give birth in May and June to the kids after 170 days' gestation. Subsequently, they form larger female-kid groups. Mature adult males (8-9 years) remain alone most of the year, joining the groups in the late summer in time for the autumn rut.

Rutting behavior is spectacular, with adult males chasing the younger males from the groups. The animals are often seen chasing each other at breakneck speeds up sheer rock faces, covering hundreds of meters' elevation in a few seconds³² (Figure 50-1).

In a study in the Bavarian Alps, home range size varied from 144 to 635 hectares (mean, 398 ha). However, the core areas (90% utilization densities) were only 4 to 7 ha in size. Females and males had similar home ranges, with home range size decreasing in males with age.¹⁰

ANATOMY AND PHYSIOLOGY

The general anatomy of the chamois is similar to the domestic goat. In the chamois there is a slight sexual dimorphism, with males weighing 35 to 50 kg (77-110 lb) and females weighing 25 to 35 kg (50-77 lb). In comparison, the southern chamois (*R. pyrenaica*) is



Fig 50-1 Alpine chamois (*Rupicapra r. rupicapra*) chasing through the snow during rut, at the Salzburg Zoo, Austria. (See Color Plate 50-1.)

somewhat smaller and lighter in weight. During summer the coat is light brown to brown with distinct, very light, yellow-white markings on the cheeks, dorsal nose, and upper neck, leaving the eyes masked in dark brown. During the winter the hair coat markedly darkens. The southern chamois has a generally lighter hair coat with a reddish tinge.

The sharply pointed, backward curved horns are found in both genders and may reach 350 mm (14 inches) in length.²⁸ The southern chamois has slightly shorter and thinner horns that are less curved. With experience, the horns may be used to determine age. Dentition is equivalent to domestic ruminants, with 32 permanent teeth. Age determination using dentition is very imprecise and varies greatly in the literature. Incisor 1 (I1) erupts at 16 to 26 months, I2 at 26 to 30 months, and I3 at 32 to 44 months, with permanent dentition established by month 45.²⁸

FEEDING AND HOUSING

Chamois are rarely held in International Species Information System (ISIS) member institutions; only 55 *R. rupicapra* in 12 institutions are listed. Munich Zoo in Germany is the only institution in the world that holds a captive group of *Rupicapra p. ornata*.³ On the other hand, the chamois is a common captive species in various game parks and nonscientific institutions throughout its European distribution range.

Generally, chamois are considered difficult to house in zoologic institutions. They are very sensitive to high environmental temperatures, but are resistant to very

low temperatures. Within their distribution range, they may be housed outdoors without a stable or additional protection. The animals are best housed as a harem group in a large, naturalistic enclosure that provides sufficient cover and shade.²⁰ The enclosure should obviously reflect the natural environment and provide rocky faces and outcrops the animals may use for climbing and viewing. Fencing of 2.5 to 3.0 m (~8-10 ft) in height is reported to be sufficient.³⁶

From the management point of view, it is important to have a plan in place to deal with juvenile males. At 2 years of age, it will be necessary to remove these animals from the group before the autumn rut because the dominant male will inflict severe traumatic injuries or death. Some institutions have reported problems with particularly aggressive males that attacked and killed females within the group. Experience has shown that these males are best removed. Introduction of new individuals into an established group is extremely difficult because of intraspecies aggression and must be carried out cautiously. Several mixed-species enclosures with chamois have been described (e.g., Salzburg with chamois, European otter, and alpine marmot). Because of the distinct fighting behavior of the chamois, it is advised not to mix this species with other mountain ungulates (e.g., ibex).

In the wild the summer diet consists mainly of grasses, browse (shrubs, trees, conifers), and a significant wooden fraction. In winter, when grasses become unavailable because of snow-cover browse becomes the main constituent.⁶ It must be assumed that foraging habits in the wild vary significantly with habitat use. Furthermore, it is important to realize that in chamois, as with many northern species, body condition has a marked annual pattern. This variation is an expression of seasonal fluctuations in metabolic rate, which enables northern wild ruminants to deal with the energetically critical winter period.⁵ Captive chamois should be provided very-good-quality hay (hand-processed, first-cut alpine hay). Additionally, browse should be available at all times. Although not necessary, small quantities of fruit and vegetables may be supplied to provide variety. As in other ruminants, local deficiencies in mineral content may be provided for with commercial salt blocks.

REPRODUCTION

The chamois have a distinct mating season. The single kid is born after 153 to 180 days of gestation in May and June in the alpine region. Only very rarely are twins born. Before birth the females no longer tolerate the

juveniles from the previous year and sometimes leave the group. Birth within the group is strongly recommended. Kids weigh between 3 and 5 kg (6½-12 lb) at birth and double their weight after 3 weeks.³⁶ Breeding generally is not a problem in this species. Pregnancy diagnosis is best achieved by determining fecal estrogens and pregnancies.³⁸

RESTRAINT AND HANDLING

Physical restraint may only be recommended for newborn animals. In the wild, chamois may be captured using box traps, foot snares, drive nets, and drop nets. Care must be taken with snares during the winter months because these may cause severe digital frostbite. Use of nets and box traps has resulted in skewed gender ratios, with significantly more females captured. Finally, it is important to realize that the effort needed to capture this species in an alpine environment is considerable. In the Swiss National Park, more than 200 hours were needed per captured animal, with 100 traps set simultaneously.⁴⁰

Chemical Restraint

Oral Sedation

Varying degrees of sedation may be achieved using acepromazine as granules (Vetranquil 1%, Albrecht, Germany) or as a paste (Sedalin, Chassot, Switzerland) at a dosage of 1 to 2 mg/kg orally (PO). The granules are mixed into moistened pelleted feed. Although this is not sufficient for subsequent physical restraint, it has proved valuable as transport and preimmobilization sedation.

Long-Acting Neuroleptics

Whereas the use of the short-acting neuroleptic acepromazine has been reported in conjunction with drive-net capture events, the use of long-acting neuroleptics has not been reported in chamois.

Immobilization

Various methods have been described to immobilize chamois chemically. In the German-speaking areas of Europe, the Hellabrunner mix (125 mg xylazine + 100 mg ketamine per mL) was the method of choice until recently. It is important to note that the required dose

(0.27 mg/kg ketamine + 0.34 mg/kg xylazine) for chamois is extremely low compared with other mountain ungulates.⁴² The advantage of this combination is that very low dart volumes (0.04-0.08 mL) are possible.

The combination of medetomidine (Zalopine, Farnos, Turku, Finland) and ketamine (Ketamidol, Richter Pharma GesmbH & CoKG, Wels) has proved to be a superior choice. In captive chamois, 0.06 to 0.08 mg/kg medetomidine with 1.5 mg/kg ketamine is recommended.⁴⁰ In wild chamois, 0.08 to 0.1 mg/kg medetomidine with 1.5 mg/kg ketamine is recommended to reduce the time to recumbency. Medetomidine is reversed with atipamezole (Antisedan, Farnos, Turku, Finland) at 0.4 mg/kg.⁴¹ Other combinations used in the past are etorphine-acepromazine, carfentanil-xylazine, and tiletamine-zolazepam.

For prolonged procedures, intubation and inhalation anesthesia with isoflurane is recommended.

Conditions requiring surgical intervention are similar to those reported in domestic goats. Intraspecies trauma with various degrees of lacerations may play an important role in chamois, and a review of the social structure is always warranted.

DIAGNOSTICS

The diagnostic approach in chamois employs the same techniques that are used in domestic goats. For an overview, the reader is referred to one of the standard veterinary texts.¹⁷ Several diagnostic limitations may result from the necessary chemical restraint.

The jugular vein is easily raised with digital pressure, allowing intravenous (IV) injection and venous blood collection. Peripheral venous catheters are placed into the jugular vein either directly through the skin or after a small skin incision. Catheters should be affixed with a skin suture securely tied around the catheter or with a drop of acrylate glue. Arterial blood collection is best carried out using either the brachial, femoral, or auricular arteries.

Using techniques described for domestic goats, cerebrospinal fluid (CSF) may be collected from the lumbosacral site. About 1 mL CSF per 5 kg of body weight may be safely removed. CSF flow is enhanced through compression of both jugular veins. Because changes in peritoneal fluid occur rapidly in response to inflammatory processes involving the peritoneum and the gastrointestinal tissues, collection and evaluation of peritoneal fluid after ultrasonographic localization of fluid pockets through abdominocentesis is an informative procedure.

DISEASES

Endoparasitic Diseases

Various *Eimeria* spp. specific to chamois have been described. Clinical intestinal coccidiosis is mostly a disease of chamois kids. A massive ventral neck edema is often observed in kids with clinical coccidiosis. In the captive environment, however, regular systematic fecal examination of the entire group for coccidia is good practice. Control of coccidia is best accomplished with toltrazuril, 20 to 30 mg/kg PO (Baycox 5% Oral Suspension, Bayer, Leverkusen, Germany). Extra-intestinal coccidiosis has been previously described in a captive chamois.¹² The presence of *Sarcocystis* spp. without any inflammatory signs is often seen at necropsy in chamois.²⁶

Liver flukes are rarely seen in chamois. *Fasciola hepatica* has been described occasionally in Austria and Bavaria. *Dicrocoelium dendriticum* is seen somewhat more frequently, but for the most part is not clinically relevant.

Various cestodes from the family of the Anoplocephalidae are regularly demonstrated. *Moniezia expansa* appears to be the most frequent cestode found in chamois. Similar to the coccidia, cestodes are encountered predominantly in kids but are associated with clinical disease in only a minority of cases. The chamois is an intermediate host for numerous canine cestodes, the most common being *Cysticercus tenuicollis*. In some areas, up to 20% of the hunted animals had *Taenia hydatigena* cysts. The most dramatic clinical cases are a result from an infection with *Coenurus cerebralis*, the *Taenia multiceps* larvae. The cyst develops in the brain and results in massive central nervous system (CNS) symptoms.

Lungworms are extremely common in chamois. In a recent study in Austria, larvae of lung nematodes were present in 86% of the samples. In most cases (85%), Protostrongylidae (small lungworms) were detected. *Dictyocaulus* spp. (big lungworms) had a prevalence of 8%. A marked seasonality in parasitologic output was noted; larval output of lungworms was significantly higher during the winter season.²⁶

Similarly, the prevalence of gastrointestinal nematodes is very high; eggs from the family of Trichostrongylidae are detected most often. Similar to the situation in lungworms, a marked seasonality in parasitologic output is noted. In contrast to the lungworms, a significantly higher nematode egg output occurs during the warm summer months than during the winter and spring months.

The reader is referred to comprehensive reviews of chamois parasites for more specific information.¹¹ Treatment of parasitic diseases in captive chamois is best accomplished using protocols developed for domestic goats.

Infectious Diseases

As they share pastures with domestic sheep and goats, wild chamois may extend the host range for a number of infectious agents. The chamois (and ibex)–domestic ungulate interface in the European Alps is an important source for potential conflict between various stakeholders, such as hunters and shepherds. The recent Office International des Epizooties (OIE) recommendations to include wildlife in the surveillance of reportable diseases highlights this problem.²

Respiratory Tract

Respiratory diseases are considered the most important pathology in wild chamois. These occasionally cause epizootics with significant die-offs. *Mannheimia haemolytica* (formerly *Pasteurella*) is the etiologic bacterial agent most frequently isolated. It is assumed that pneumonia-related die-offs are multifactorial in development, and several viral agents have been described. Chamois have been reported to be seropositive for bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), and bovine respiratory syncytial virus (BRSV) during an outbreak of respiratory disease in Italy.^{14,43} In addition to viral and bacterial agents, respiratory disease is associated with lungworm infestations. The postmortem examination of chamois during a respiratory disease die-off revealed that 14 of 18 animals with lung lesions also had a verminous pneumonia associated with *Protostrongylus* spp.¹⁴

A recent survey in a national park in Austria demonstrated that in 75 of 95 examined chamois, pathologic pulmonary lesions associated with *Protostrongylus* spp. were present.²⁶ Recently, the unclassified bacterium cilia-associated respiratory (CAR) bacillus was demonstrated in the respiratory tract of wild chamois from northern Italy.⁸ Additionally, a case of fibropurulent bronchopneumonia associated with *Moraxella bovis* has been described.³⁰

Infectious Keratoconjunctivitis

Infectious keratoconjunctivitis (IKC) is a condition caused by *Mycoplasma conjunctivae* that affects the eyes

of domestic goats and sheep. It is distributed world-wide. In the European alpine region and the Pyrenees, chamois are frequently affected. The disease was first described in chamois at the beginning of the twentieth century.³⁹ Whereas IKC in domestic Caprinae has only a moderate impact, it has fatal consequences for wild ungulates. Mortality is reported to reach 30% in some outbreaks, having a significant effect on demography.¹⁶

The disease is characterized by unilateral or bilateral conjunctival and corneal inflammation. With disease progression to a mucopurulent conjunctivitis and subsequent ulceration, the cornea becomes opaque and may perforate, rendering the animals totally blind (Figure 50-2). Blind chamois in the wild demonstrate a dramatic circling behavior and are often found in deep, round snow pits.²⁴

Detection of the infectious agent using classic culture methods is difficult and requires specialized expertise. A recent polymerase chain reaction (PCR) assay allows rapid diagnosis.²⁵

Recovery from the disease is the most prevalent course. Particular attention should be given to avoiding disturbances in areas affected by an IKC outbreak. Individual animals that are obviously blind and suffering should be shot. Whereas wild animals should not be treated, captive animals may benefit from systemic and topical antibiotics. Treatment with a long-acting oxytetracycline (20 mg/kg intramuscularly) formulation in conjunction with topical chlortetracycline has been suggested.

Disease prevention centers on preventing spillover of *M. conjunctivae* from domestic Caprinae on the shared alpine pastures by limiting salt licks and thus the frequency of encounters. In the case of a disease outbreak in the captive environment, it may be bene-

ficial to isolate affected animals from the group and initiate prophylactic treatment with oxytetracycline. Before adding new animals to a group, conjunctival swabbing appears prudent.

Other Infectious Diseases

Recently, *Pestivirus* infections have been identified in southern chamois from Spain and France.²⁷ The affected animals showed nonspecific CNS symptoms such as motor-locomotion impairments and an absence or reduction in flight response to humans. Serologic examinations have demonstrated a high prevalence of pestiviruses in clinically inapparent populations in Spain. Because of the important economic losses worldwide caused by *Pestivirus*-associated diseases (e.g., BVD, border disease virus, classic swine fever virus), intensive monitoring and characterization of the pestiviruses in the chamois populations throughout the distribution range are ongoing.²¹

Disease associated with and isolation of *Brucella melitensis* from chamois have often been described throughout the distribution range. Animals with symptoms present with the classic signs of polyarthritis and orchiepididymitis.²³ As with other diseases described here, infection with *Brucella* spp. has potential economic implications resulting from the extensive chamois–domestic ungulate interface. As a differential diagnosis, clinicians must consider pyogenic arthritis caused by *Actinomyces pyogenes*, as previously described in a wild chamois in Spain.²⁹

Various other infectious diseases have been reported in chamois on a case study basis. When establishing differential diagnoses, it is advisable to consider diseases described for domestic goats and sheep.



Fig 50-2 Chamois with mucopurulent conjunctivitis and opaque cornea from infectious keratoconjunctivitis (IKC). (See Color Plate 50-2.) (Courtesy Centre for Fish and Wildlife Health, University of Bern, Switzerland.)

Skin Diseases

Scabies

Sarcoptic mange is a contagious parasitic skin disease caused by *Sarcoptes scabiei* that affects a wide range of domestic and wild species. The disease in chamois was initially described in the Austrian Alps at the beginning of the twentieth century.¹⁹ It has been postulated that the disease initially spread from this area. Presently, mange is considered endemic in the eastern alpine chamois populations in Austria, Italy, Germany, and Slovenia and sporadically is the cause of significant mortalities. The disease has never been described in the central and western alpine regions (Switzerland, France). Recently, a geographically limited but severe

outbreak was described in the southern chamois in northwestern Spain.¹⁸ The occurrence of scabies in wild populations is cyclic, with peaks every 7 to 15 years in accordance to the area. Various multifactorial causes for the outbreaks have been discussed.²²

In chamois, clinical scabies typically begins at the head and neck and then spreads over the back (Figure 50-3). In severe cases the entire abdomen and the extremities are also affected. Histopathologic findings include orthokeratotic and parakeratotic hyperkeratosis, with epidermal hyperplasia, crusting, and exudation. Transmission of mites from naturally infected goats with the development of disease in chamois has been demonstrated.³¹ Diagnosis is made by demonstrating mites in skin scrapes and biopsies. Recently, a sensitive and specific enzyme-linked immunosorbent assay (ELISA) was developed that allows the detection of antibodies in serum samples. This test also enables detection in asymptomatic carriers, so it is hoped that it will significantly improve disease-monitoring possibilities in the endemic areas.³⁷

As in domestic livestock, scabies in captive chamois is easily treated with ivermectin, 0.2 mg/kg subcutaneously.

Scabies has zoonotic potential. Recently, an episode of human scabies of animal origin (pseudoscabies) was described in seven people involved in the capture of infected chamois.³³

Dermatophilosis

Dermatophilosis, a suppurative, inflammatory skin disease caused by the actinomycete bacterium *Dermatophilus congolensis*, has been reported repeatedly

from various alpine regions. In a postmortem survey of respiratory disease, concurrent dermatophilosis was noted in 11 of 18 animals examined.¹⁴ The ear, muzzle, face, and tail are common locations for this disease. Dermatophilosis is diagnosed by demonstrating rows of coccoid bodies with a "railroad track" appearance on a Giemsa-stained smear.

It is speculated that clinical disease results from a combination of predisposing factors, such as stress and concurrent infections. In captive animals, systemic procaine penicillin G (20,000-70,000 units/kg) or oxytetracycline (20 mg/kg) in combination with symptomatic topical treatment (CuSO_4 or ZnSO_4) has been suggested. Because of the zoonotic potential of dermatophilosis, adequate care during treatment is warranted.¹

Contagious Ecthyma

Contagious ecthyma (CE, orf) is a pustular dermatitis caused by a parapoxvirus. CE has been reported periodically in wild and captive chamois. The pustular and vesicular lesions are predominantly seen on the mouth and face but may also occur on the genitalia and feet (Figure 50-4). In most cases the lesions resolve without treatment after 2 to 3 weeks. Domestic sheep and goats are viewed as a reservoir. In the event of an outbreak, the diseased animal should be isolated from the group (beware of fomite transmission).

Only in animals with severe CE is symptomatic topical treatment indicated. A commercial vaccine is available, and its use would be warranted in managing an infected group. CE has a high zoonotic potential, causing lesions on the hands and fingers, and therefore necessitates adequate hygienic precautions.¹



Fig 50-3 Chamois with clinical scabies. The typical lesions begin at the head and neck and then spread over the back. (See Color Plate 50-3.) (Courtesy T. Steineck, Research Institute of Wildlife Ecology, VMU, Vienna.)



Fig 50-4 Chamois with contagious ecthyma. The pustular and vesicular lesions are predominantly seen on the mouth and face. (See Color Plate 50-4.) (Courtesy Centre for Fish and Wildlife Health, University of Bern, Switzerland.)

Infestation

Infestations in chamois with *Trombicula* spp. and a variety of ixodid ticks have been described.

Neoplasms

Only three cases of tumors in chamois have been reported in the literature. Most interesting is the description of adenomatous neoplasms of the gall-bladder in 5 of 64 chamois examined from a national park in Italy. A cause for this frequency could not be determined, but the authors speculated that an environmental contaminant might be responsible.⁹ Furthermore, a fibroblastic osteosarcoma and a thalamic astrocytoma have been described in the chamois.^{15,35}

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Color Plate 50-1 Alpine chamois (*Rupicapra r. rupicapra*) chasing through the snow during rut, at the Salzburg Zoo, Austria. (For text mention, see Chapter 50, p. 409.)



Color Plate 50-2 Chamois with mucopurulent conjunctivitis and opaque cornea from infectious keratoconjunctivitis (IKC). (For text mention, see Chapter 50, p. 412.) (Courtesy Centre for Fish and Wildlife Health, University of Bern, Switzerland.)



Color Plate 50-3 Chamois with clinical scabies. The typical lesions begin at the head and neck and then spread over the back. (For text mention, see Chapter 50, p. 413.) (Courtesy T. Steineck, Research Instructor, Wildlife Ecology, VMU, Vienna).

Color Plate 50-4 Chamois with contagious ecthyma. The pustular and vesicular lesions are predominantly seen on the mouth and face. (For text mention, see Chapter 50, p. 413.)
(Courtesy Centre for Fish and Wildlife Health, University of Bern, Switzerland.)



CHAPTER 51

Gastrointestinal Nematodiasis in Hoofstock

EDMUND FLACH

Species of perissodactylid and artiodactylid ungulates evolved to take advantage of the grasses, shrubs, trees, roots, tubers, and fruits on and below the surface of the land. Similarly, nematode parasites evolved to take advantage of the mucosal surfaces of the ungulates' gastrointestinal (GI) tracts, to maximize the chances of their eggs and larvae surviving outside the host and infecting a new host.

This chapter examines the problems that occur when the host-parasite balance tips in favor of the nematodes and how we can investigate and control, or live with, the situation.

BACKGROUND

All hoofstock, especially those with access to grass enclosures, should be assumed to carry a range of GI nematodes. The extent of the infestation, the species of nematodes involved, and the clinical significance should be assessed for each host species.^{6,7,12}

In which climatic region is the ungulate collection? In a temperate zone, nematodes would be expected to thrive in the wet summer but would need to survive a harsh winter as resistant stages in the environment and within some of the hosts. In Mediterranean and semiarid regions, however, the cool, wet winters would favor the nematodes, but they would be suppressed and would need to survive during the hot, dry summers.

What ungulate species are held by the collection? Species generally evolved with the nematodes that survive in the same climatic region and would be expected to be relatively resistant to clinical disease, certainly compared with species from different climates. In the temperate climate of the United Kingdom, species of deer such as red deer (*Cervus elaphus*) show little clinical effects of nematodiasis unless circumstances favor the nematodes (see later discussion). However, antelope such as scimitar-horned oryx (*Oryx*

dammah) and addax (*Addax nasomaculatus*), from semi-arid and arid regions, and musk ox (*Ovibos moschatus*), from the tundra, are highly susceptible.

What do the clinical and necropsy records reveal? Even when no specific parasite monitoring has been done, the general records for the collection should still have much useful information. How long have species of interest been in a particular enclosure? How has the herd size and stocking density changed over time? Have there been reports of poor body condition, diarrhea, or deaths in the group? Are there fecal egg count results on file?

CLINICAL PRESENTATION

Primary Disease

Under certain situations, GI nematodes may cause clinical disease without other pathologic agents. Examples include the following:

- In haemonchosis, *Haemonchus contortus* may cause severe damage to the mucosa of the abomasum of ruminants, or the third compartment (only one stomach, but three compartments) of camelids, resulting in gastric hemorrhage. Heavy infections may result in sudden death.¹⁴
- Type I ostertagiasis is caused by the smaller abomasal nematodes of the genus *Ostertagia*. Closely related genera such as *Telostertagia*, *Skrjabinagia*, and *Camelostrongylus* may also cause extensive damage to the abomasal wall. This results in leakage of proteins and thus an increase in plasma pepsinogen, increase in protein turnover, and eventually a hypoproteinemia and change in pH. Heavy infections tend to occur in young calves in their first season on grass, resulting in diarrhea, poor growth, and even loss of body condition and death. In temperate climates, this occurs at the end of the summer.

- Type II ostertagiasis has been described in domestic cattle. However, the synchronized development of arrested larvae in the abomasal wall after a stressful event has been reported in an adult male blackbuck (*Antilope cervicapra*) in late winter after fighting with another male.²
- Trichuriasis has been shown to cause colitis in camels (*Camelus* spp.)⁵ and often infects giraffe (*Giraffa camelopardalis*).
- Ascariasis has been responsible for the death of zebra foals, usually from blockage of the intestines by large accumulations of adult *Parascaris equorum*.¹⁰ Another ascarid, *Toxocara vitulorum*, is transmitted in the colostrum from cattle to calves and may be pathogenic in neonatal European bison (*Bison bonasus*).

In many cases, more than one species of nematode may be found, or nematodes and other parasites are found together, and it may be impossible to decide which species is the main pathogen. For example, a mixed infection with a *Trichuris* sp. and several *Eimeria* coccidial spp. was thought to be responsible for diarrhea in a 10-day-old red lechwe calf (*Kobus leche leche*).³

Secondary Disease Factors

Secondary disease caused by GI nematodiasis is probably more common than primary disease and likely underreported. In these cases, many pathologic processes may be causing loss of body condition, weakness, and death, and the relative importance of each factor may be difficult to determine. Common factors in secondary disease include climate, malnutrition, stress, physiologic state, and concurrent disease.

Climate

In temperate regions, animals need to expend more energy to maintain their core body temperature in winter. Species that have evolved to survive winters with thicker coats or insulation will require less energy than species from other regions; even for these animals, however, extended periods of rain may saturate coats and make them less insulating, leading to increased heat loss. In these circumstances, animals may be in negative energy balance for extended periods, and any additional drain on their resources, including parasitism, may be fatal. The increased mortality of deer in winter is recognized as *winter death syndrome*. Hot and dry summers may be equally stressful to species not regularly exposed to such climate.

Malnutrition

Moderate levels of parasitism may be tolerated by hosts if they have plentiful food of good quality. Similarly, the extra energy requirements during cold weather may be met by increased food intake. There are limits, however, and animals may not have sufficient appetite to eat the extra food needed, or GI nematodiasis may depress appetite. Therefore, food of higher energy density must be offered to avoid a negative energy balance at these times. In hoofstock, this can be dangerous because high-energy food may cause acidosis (in ruminants), laminitis (in Perissodactyla), and other metabolic problems.

In addition to the supply of energy, which may be met by carbohydrate, fat, or protein in the diet, GI nematodiasis causes an increased turnover of protein that must also be matched by dietary input.

The intake of vitamins and minerals is important for the general health of any animal, but among hoofstock, copper deficiency may be particularly relevant. At Whipsnade Zoo, London, yak with low blood copper concentrations were found to harbor significant nematode burdens despite regular deworming.⁴

Browsing herbivores ingest plant tannins, which are thought to play a role in suppressing nematode infections. The effect has been also been shown in domestic sheep.¹

Stress

Besides initiating type II ostertagiasis, as previously mentioned, stress also has a general effect on immune status. What constitutes a stressor will vary between species and individuals and may not always be apparent. Overcrowding is likely to be apparent, but even when the enclosure density is adequate, the makeup and dynamics of the group may be stressful. For example, leaving more than one adult male of certain species with a group of females may or may not result in overt aggression and fighting, but in either case may result in heightened stress in the group, males and females alike. Thus, chronic GI parasitism in Thomson's gazelles (*Gazella thomsonii*) at Whipsnade was controlled better by removing all except for one male than by regular anthelmintic treatments.⁹

Physiologic State

Growth, pregnancy, and lactation all require increased energy and protein intake. Therefore, growing animals and pregnant or lactating females are more likely to be affected by heavy parasitism than adult males and nonbreeding females.

Concurrent Disease

It is rare for animals to encounter only one pathogen at a time. If the animals are overcrowded and the enclosure is heavily contaminated, they will be challenged with high doses of viruses, bacteria, and fungi in addition to the nematodes and other parasites. Even in less severe situations, infection with one pathogen may weaken an animal and make it more susceptible to another pathogen. In these cases the GI nematode infestation may predispose the animal to a secondary infection, or nematodes may find it easier to establish in an animal already weakened by a different primary disease.

DIAGNOSTIC INVESTIGATIONS

The level of investigations will depend on the number of species and individuals involved, the severity of the problem, and the resources available. However, consideration should be given to the diagnostic approaches discussed next.

Clinical Examination

Clinical examination may be more difficult for many large ungulates and those in herds. However, some collections have physical restraint systems that allow close inspection, sample taking, and treatment of deer and antelope. If these are not available, examiners should still be able to observe herds at feeding sites (Figure 51-1) and then select a few individuals to assess while under chemical restraint.

A full clinical examination should be carried out, including measurement of body weight, assessment of body condition, mucous membrane color, state of hydration, and a systematic check of all body systems. Other common causes of loss of body condition, which therefore should be considered along with nematodiasis, are poor dentition, malnutrition, chronic bacterial infections such as tuberculosis and paratuberculosis (Johne's disease), and liver, kidney, and heart failure. Alternative causes of diarrhea include enteritis from viral, bacterial, and protozoal infections; malnutrition; and toxicosis.

Fresh feces, preferably from the rectum, should be collected from the individual(s) and tested for bacterial pathogens and parasite ova. In addition, routine hematologic and biochemical tests, plus trace elements, should be performed. Plasma from ruminants should be tested for plasma pepsinogen as an indicator of parasitic abomasal damage. (Normal concentrations will vary with the laboratory, but at Whipsnade we consider any result less than 2 U/L at 37° C as normal.)

Group Fecal Egg Counts

Egg counts are the most useful results for investigating and monitoring GI nematode infections. The test is simple, inexpensive, and gives a reasonably accurate measurement of infection level. To maximize the test's potential, however, it is important to know some of its limitations.

The test relies on the different densities of nematode eggs and other fecal matter and uses a concentrated solution, usually of a salt but occasionally sugar, to float



Fig 51-1 Herd of nilgai (*Boselaphus tragocamelus*).

eggs while everything else sinks. By using a known concentration of feces (e.g., 3 g in 45 mL) and a known volume under the grid of the McMaster slide, the number of eggs per gram of feces may be calculated. However, some fecal samples may contain light, fibrous debris that also floats. If this is a problem, the feces may be suspended initially in water, centrifuged, and the pellet resuspended in the flotation solution.

A normal flotation solution is saturated sodium chloride, which is also easy to obtain. Heavier eggs, such as *Trichuris* spp., may not float well in this solution, however, and a denser solution may work better, such as commercially available silver nitrate solution (Ovassay Plus, Synbiotics Corporation, San Diego) or a homemade solution of zinc sulfate with specific gravity of 1.3 (~580 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ per liter, but check with a hydrometer). Other parasite eggs, such as the liver fluke *Fasciola hepatica*, will also float in these solutions.

Whatever technique is chosen, it is important to keep using the same technique because it is not the one result that is important (except for clinical assessment of an individual), but rather the range of results within a herd and over time. Therefore any errors within the test, provided they are consistent, will be the same each time and will not affect the differences between results.

The ideal situation for monitoring a herd of ungulates would be to test the feces of each individual on each occasion, preferably collected from the rectum or at least freshly voided. This is unlikely to occur unless the collection has either a handling system through which the herd passes regularly or sufficient staff and time to watch each individual defecate. Most situations demand a compromise by examining either (1) a number of samples from the herd and recording the median, quartiles, and range or (2) a mixed bulk sample containing pellets from many different fecal piles. The first method provides much more information about parasitism in the group, but requires that samples be stored and tested individually.

It is important that the sample collector understands the reasons for the monitoring so that the person may (1) identify and collect the correct feces for the species of interest, (2) collect the correct number of samples and record information on the sample pot(s), and (3) record, and collect separately, any feces of unusual consistency.

When calves are present in the herd, it may be beneficial to request that calf feces be collected and tested separately. There may be high numbers of eggs in calf samples, but if only one or two are collected and mixed with several adult samples, the bulk fecal egg

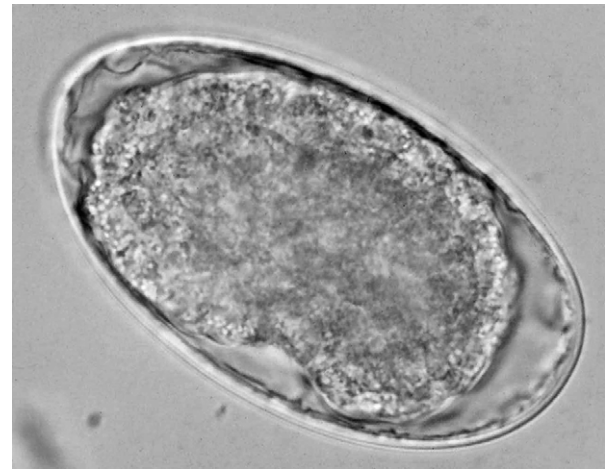


Fig 51-2 Typical trichostrongyle-type egg. (See Color Plate 51-2.)

count may stay below a threshold level set for initiation of treatment (e.g., 200 eggs per gram).

The speciation of nematodes based on the appearance of fecal eggs is rarely possible, but counts should be expressed separately for the morphologically distinct egg types: strongyle (or trichostrongyle [Figure 51-2]), *Nematodirus* spp., *Trichuris* spp., *Capillaria* spp., and ascarids. Further identification is usually done on adult parasite collections postmortem, although some differentiation of eggs may be achieved by measuring large numbers or by allowing them to hatch in cultured feces and then examining the third-stage larvae.¹³

Monitoring the egg counts over time can indicate the seasonality of infection, the impact of reproductive events (e.g., periparturient increase in fecal egg counts from adult females, increased counts when young calves are starting to graze), and the effectiveness of any anthelmintic treatments. Again, a compromise often needs to be made between the need for maximum information (frequent sampling throughout the year) and the costs (time and finance) of collecting and testing samples. The fecal sampling protocol at Whipsnade is based on an assessment of risk and severity of parasitism for each species of ungulate. Thus, common hippopotami (*Hippopotamus amphibius*) are not routinely sampled because we have never encountered a case of GI nematodiasis in the species, whereas the herd of scimitar-horned oryx, when breeding, is sampled every 4 weeks.

Pasture Larval and Soil Egg Counts

There are techniques for counting the numbers of infective third-stage larvae on pastures, as well as for detecting nematode eggs in soil and other substrates. Although time-consuming, these techniques

are essential for any comprehensive study of nematode infections in a particular host and environment.

Necropsies

Necropsy examinations should be carried out on all ungulates that die in zoos and a proportion of those that perish in extended-area or wild situations. In addition to general findings about the animal's condition, the disease processes present, and the likely cause of death, the examination must specifically focus on GI parasites and their effects (e.g., parasitic nodules in abomasum of ruminants, verminous arteritis in equids).

The entire GI tract needs to be isolated and, if possible, individual parts tied off with string. If other samples are needed, such as small intestine contents for clostridial enterotoxins, small parts of the tract may be tied and opened separately. The major sections of the GI tract; stomach (abomasum in ruminants, third compartment in camelids), small intestine, cecum, and colon (usually together, but kept separately if appropriate) are then opened in buckets so that the contents may be collected, representative pieces collected for histopathology, and the entire mucosa examined under a gentle flow of water, with the washings added to the contents in the bucket. More water is added up to a known volume (e.g., 10 L for deer and antelope, but more for large ungulates), and then an aliquot is collected for later parasite counting and identification. A 2% aliquot works well, but if large numbers are expected, a 1% or even a 0.5% sample may be taken. If the carcass is freshly dead, the samples of contents should first be refrigerated for 24 hours to kill the nematodes, then half the water replaced by 10% formalin. If the carcass has already been refrigerated, the formalin may be added immediately. It is not necessary to add formalin if the samples are to be examined immediately; the nematodes may then be removed into 70% alcohol for storage, but this is rarely possible in most zoo and wildlife situations. Feces should always be collected from the rectum and a fecal egg count performed.

The samples of contents are examined in a Petri dish under a dissecting microscope, and all nematodes, plus any other parasites, are removed and counted. These should then be sent for identification. In-house examination is facilitated by starting with a list and key for the nematodes already known to occur in the collection or area, then concentrating on examination of males because of their distinctive spicules (Figure 51-3). Unknown males and any females of particular interest may be sent to a specialist center.

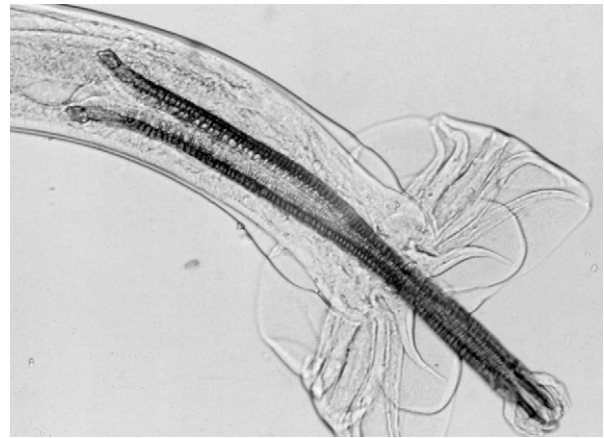


Fig 51-3 Caudal end and characteristic bursae of *Camelostrongylus mentulatus*, a common abomasal nematode of zoo ungulates. (See Color Plate 51-3.)

Because parasite collections are time-consuming, especially isolating, opening, and washing the entire small intestine, the standard protocol for routine monitoring of ruminants and camelids at Whipsnade Park is as follows:

- Examine and collect from every abomasum or third compartment; this is easy and quick to do, and most severe nematodiasis is centered in these areas.
- Examine several lengths of small intestine of all carcasses, but collect only from every second or third individual of the species (unless nematodiasis is suspected from gross findings).
- Examine only the cecum and proximal colon for visible nematodes, such as *Trichuris* and *Bunostomum* spp.

TREATMENT AND CONTROL

Individual animals may be treated by administering an anthelmintic by injection (if restrained or immobilized), by dart, as a pour-on preparation, or most often, in the feed. In addition, supportive therapy, especially nutritional supplementation, should be given. Repeat treatments may be necessary to cover the period of risk. Groups of animals are most likely to be treated orally, unless the collection has a handling system to which the animals are accustomed; medicated food should be fed in sufficient troughs and for enough time to ensure that all individuals in the group, especially the low-ranking animals because they are likely to have the highest infection, receive a full therapeutic dose. This is important not only for the individual but also to minimize the risk of resistance to the drug.

Common anthelmintics include the benzimidazoles (e.g., fenbendazole, 7.5 mg/kg), the avermectins (e.g., ivermectin, 0.2 mg/kg in most species and 0.4 mg/kg in deer), and levamisole (7.5 mg/kg).

Optimal control of GI and other forms of nematodiasis depends on (1) knowledge of the biology and life cycle of the species, (2) good management to ensure that the ungulate hosts may best withstand infection, (3) targeted interventions to prevent excessive buildup of infection, and (4) constant monitoring.⁸

Pasture Management

The aim should be to maintain the lowest stocking density range compatible with keeping the grass grazed reasonably short and also allowing the visitors to see enough animals. Increasing stocking density will reduce the amount of grass per individual, increase the fecal contamination of the area and thus infective larval load, and ultimately also increase stress within the herd. It is feasible to rotate paddocks between ungulate species with different parasite spectra, such as equids and bovids, but only if the enclosures are built to allow easy movement of herds between them and the housing is suitable for the different taxa. Ideally, each paddock should be left fallow for a year, although this is unlikely to be acceptable in a zoo situation.

A variety of machines may be used to remove feces from paddocks, including paddock vacuum cleaners (e.g., Trafalgar Cleaning Equipment, Horsham, West Sussex, U.K.), but these are usually limited for use with larger fecal pellets (e.g., horse) and when the paddock is dry enough to allow heavy machinery. Also, excessive removal of feces could be stressful to ungulate species that defecate in piles as a way of marking territory.

Nutrition

As mentioned earlier, malnutrition may be a factor in allowing nematodiasis to become a clinical problem. Therefore, it is essential that herds are fed sufficient food of adequate quality and in such a way that all individuals in the herd may have access. Concentrate pellets should be fed off the ground so that animals are not ingesting contaminated soil with the pellets, and the containers used, normally food troughs, should be cleaned regularly. When grass is limited, good-quality hay should be provided, and vitamins and minerals should be supplemented as required.

Stress

Reduce stress by keeping the stocking density low, removing subadult males before they start to test the dominant male, and avoiding the mixing of compatible species.

Routine Anthelmintic Treatment

Some prophylactic anthelmintic treatment will be necessary for many ungulate species kept in captivity, but only for those at risk of clinical disease. Development of anthelmintic resistance is a real risk in nematodes in zoos unless great care is taken in how these agents are used.

Treatments should be targeted to specific times of year when anthelmintics will have maximum benefit in protecting susceptible animals from clinical disease and reducing the main fecal shedding of eggs. This might include a spring treatment to reduce the fecal rise in eggs from periparturient females, one or more treatments in summer to protect calves and reduce their massive egg output, and a late-autumn treatment to kill arrested larvae within hosts. For more susceptible species, it might be necessary to treat every 3 to 4 weeks from spring through autumn, but in other cases, if a herd is not breeding one year, for example, the number of treatments should be reduced or eliminated.

An alternative approach is to treat only if fecal egg count rises above a threshold, but this carries the risk of missing highly infected individuals, such as calves, until it is too late.

Whatever frequency of treatment is chosen, it is vital to treat every individual in the herd with the full therapeutic dose of anthelmintic to avoid the risk of selection for resistance. If herd members are fed individually, the correct dose may be fed to each, but more often the drug must be added to the concentrate pellet and fed to the whole herd. For the last 10 years at Whipsnade, we have used a medicated grazer pellet (14% crude protein) and fed this at normal feeding rates for 3 consecutive days. The drug inclusion rate is determined by the daily intake of pellets, which we have estimated as 1% of body weight. Some species eat less than this and will not receive the therapeutic dose on one day but should be sufficiently treated over the 3-day course. Almost all ungulate species have eaten the pellet, even if it is not their normal concentrate, so we have been able to order medicated feed in bulk. Two anthelmintics are alternated: fenbendazole one year and ivermectin the next year. In addition, the final treatment of the year for equids is an unrelated compound, pyrantel embonate.

A drug from a third, unrelated class of anthelmintics may be added and used in a 3-year cycle. One U.K. collection treats with two different anthelmintics at the same time, drawn from four different classes of drugs.¹¹

ANTHELMINTIC RESISTANCE

Resistance should always be suspected if (1) clinically affected ungulates do not respond to treatment; (2) fecal egg counts remain high, or return in less than 3 weeks to high numbers, after treatment; or (3) large numbers of nematodes are found at necropsy in a recently treated animal. Often the cause is incorrect treatment, so it is important to supervise repeat treatment and arrange for fecal samples to be collected before and at intervals after treatment. If eggs are not eliminated by the treatment, further fecal samples should be submitted to a parasitology laboratory for egg hatch and larval development testing.

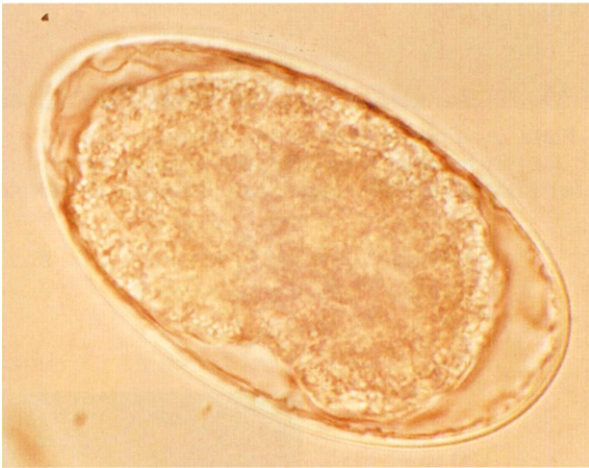
At Whipsnade, trichostrongyle-type nematode eggs from feces of scimitar-horned oryx and blackbuck were tested, but there was no evidence of resistance to benzimidazoles in either case. However, similar eggs from Prewalski's horse feces did hatch, and there was some larval development in the presence of fenbendazole, indicating a degree of resistance.

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Color Plate 51-2 Typical trichostrongyle-type egg. (For text mention, see Chapter 51, p. 419.)



Color Plate 51-3 Caudal end and characteristic bursae of *Camelostrongylus mentulatus*, a common abomasal nematode of zoo ungulates. (For text mention, see Chapter 51, p. 420.)

Tuberculosis in Michigan Deer

JAMES G. SIKARSKIE

HISTORY AND BACKGROUND

Tuberculosis (TB) caused by *Mycobacterium bovis* is endemic in the free-ranging white-tailed deer (*Odocoileus virginianus*) population in northeastern lower Michigan. The first documented case was shot by a hunter during the 1975 hunting season, an older doe estimated age 9.5 years. It had caseous abscesses in the lungs and chest cavity that cultured positive for *M. bovis*. At the time, Michigan had not yet attained “tuberculosis accredited-free” status and was in the second year of the required 5 years of no new TB cases in the state’s cattle population.

The Department of Natural Resources (DNR) and Department of Agriculture discussed the significance of this case of bovine TB in a free-ranging wild deer. It was thought that the deer must have been an orphaned fawn found and raised on raw milk by a local dairy farmer with recent *M. bovis* in cattle. Tuberculosis in free-ranging white-tailed deer was considered unlikely because the disease was believed to be associated with crowded and stressed animals in confinement. Therefore, the case was dismissed as an isolated incident, and no testing or surveillance efforts were made on wild deer in the area.

Second Case

The state of Michigan achieved “tuberculosis accredited-free” status in 1979 for its cattle population. No further cases were noted until fall 1994, when another hunter shot a deer that was diagnosed with TB in the same general area where the positive deer was found in 1975. This animal was a 4.5-year-old buck. This case was still considered an incidental finding, but this time, surveillance efforts were implemented, with testing of cattle and examination of wild deer in the area.

It was hypothesized that the deer must have contracted bovine TB from infected cattle because it was again considered unlikely that deer could sustain TB in a free-ranging population. Cattle were no longer

tested because Michigan was accredited as TB free, so regulatory officials were reasonably sure they would find the source of the disease in the cattle population.

Tuberculosis testing of all test-eligible livestock (cattle, captive deer, bison, goats) within a 5-mile (~16-km) radius of the initial 1994 deer case failed to identify a source of the infection in domestic livestock. The location of the deer with TB found in 1975 was within this test area, so it now seemed likely that TB was present in the deer and may have been for some time.

Hunter-harvested deer were tested in the initial 16-km radius during the 1995 hunting season, with 18 more positive deer found, plus a few cases in the general area around the official test area. To facilitate surveillance and management of the problem, the DNR created a new Deer Management Unit (DMU) 452 in 1996.

The history and development of this unique situation in Michigan have been analyzed and chronicled by many authors. There is a significant body of knowledge in regard to the disease caused by *M. bovis* in cervids.² One may only speculate on the origin of the *M. bovis*. However, an analysis of how the problem developed may help to explain what went wrong and to prevent the development of future problems, as well as explore methods to manage this disease in cervids.¹⁴

Efforts of Deer Management and “Clubs”

The new DMU 452 encompassed many “hunt clubs,” large tracts of privately owned land that had been bought by wealthy businessmen in the late 1800s and early 1900s to provide areas with members-only hunting privileges. These large tracts were available because the land had been cleared of trees and farmed with rather poor success; the soils were light and did not sustain agricultural crops well. Often these farms failed, and land reverted back to the state when taxes were not paid, allowing the land to be bought inexpensively by clubs.

Public lands were managed by DNR to increase the carrying capacity of the habitat for white-tailed deer with the Deer Range Improvement Program (DRIP). Deer hunting is a tremendously important contribution to Michigan's economy, so much effort and many state and federal funds go into managing the deer herd. Rules and regulations on how deer are hunted and shot (e.g., from tree stands, over bait, type of weapon, length of season) are promulgated and enforced, with zones (DMUs) and quotas determined by biologists in an effort to keep the population healthy. When a firearms deer license is purchased in Michigan, it allows the hunter to shoot an antlered buck anywhere, but antlerless deer (does and fawns) may only be harvested with an additional permit for designated DMUs. Issuance of these limited permits is based on hunting pressure and deer productivity in an effort by biologists to regulate the population within the carrying capacity of its habitat, using hunting as a management tool.

This "biologic carrying capacity" is determined by study of established wildlife management values, such as available food, cover, productivity, and winter severity. In contrast, often the hunters and private land owners apparently base decisions on the "social carrying capacity" of an area. In my view, this determination is based more on political values, such as stakeholders' attitudes, and is a more difficult figure to calculate. It is a common practice for some hunters to secure antlerless permits with no intention of taking a doe or fawn because they believe the deer population is insufficient in the area. The DNR has an even greater challenge when using hunting and other traditional management tools, such as controlled timber harvest or habitat manipulations on private lands, especially the large tracts with "limited hunting pressure" controlled by clubs. Although they did not fence their lands, the clubs controlled land use and regulated or limited hunting pressure much more than on public lands.

Additionally, many clubs baited during the fall to attract and keep deer on their land for an enhanced success rate, with less effort for their members, and to protect or keep "their" deer from being shot on adjacent public lands. Some clubs fed deer year-round in an effort to provide more and larger deer for their members. Unfortunately, more deer translates into smaller, less healthy deer when food availability is limited in quantity and quality.

It is known that adequate nutritious food is essential in the early fall to build up fat reserves to be used during the winter, when natural food and movement are limited by cold and snow. If white-tailed deer do

not have adequate fat reserves to draw on, they may not be able to eat enough during the winter to survive, even if supplements are provided and are nutritionally complete.¹⁷

Baiting and Increased Disease Risk

Congregating artificially high numbers of socially and nutritionally stressed deer over artificial food sources also created a greatly enhanced risk of disease transmission. As the population of deer grew, some clubs attained seasonal local deer densities of almost 200 deer per square mile. In an effort to increase the deer harvest, managers made the already-widespread practice of "shooting over bait" legal. Many hunters did so even if they were philosophically opposed to the concept of baiting, because deer stayed around these large bait sites on someone else's land if they did not bait on the land they could hunt.

Baiting became big business, with the demand for cull crops such as apples, potatoes, and carrots exceeding supply. Farmers raising crops for deer bait were complaining that deer were eating their crops! They applied for and received crop damage, or "depredation," permits from the DNR to shoot deer legally in the early fall before the regular gun season. They could let hunters fill these permits and charge for the opportunity to hunt on their land as a way to recoup lost revenue because of the damaged crops.

In one case, a farmer was issued a depredation permit to shoot 30 deer on his land, which he legally did by charging hunters to help reach the quota. Of the 30 deer shot, 29 were large bucks. This did remove some more deer from an already high population and compensate the farmer for some of his losses, but it did not remove the reproductive segment of the population (does), as intended by the wildlife biologists issuing the permits. Furthermore, hunters on adjacent lands during the regular season were less successful at harvesting a trophy buck and increased their baiting efforts to attract more deer to improve their chances of success.

The DNR also issued antlerless permits to increase the doe harvest during the gun season, but there is a strong cultural sentiment against shooting does by many hunters who want more deer.

Much of the baiting was being done during the critical fall period, when fat would normally be accumulated by eating more nutritious foods such as acorns and forage dispersed over large areas. These bait and feeding stations sometimes kept deer from their natural or traditional movement to winter yards, and

the artificially high densities over artificial food supplies have helped create the current problem with *M. bovis*. It is likely that TB persisted at low levels in the deer herd when the population was within the carrying capacity of the habitat but had not spread due to lower densities.

Tuberculosis may transmit vertically in smaller deer family groups and may have spread horizontally in areas (e.g., some clubs) where cattle were fed along with deer. Many of these clubs had a history of raising cattle in the 1950s, when bovine TB was known to be common in Michigan cattle.¹³ Cattle were rounded up, tested, and slaughtered, but the free-ranging deer likely perpetuated the disease after the cattle source was removed. Previously, infected livestock were considered necessary to spread TB to cervids, and it was thought that infection would not be maintained in a properly managed free-ranging population.⁴

This might have been true, but management of the white-tailed deer population in Michigan clearly was not achieving the goals of having a healthy, sustainable population kept within the carrying capacity of the habitat.

Studies show that disease may be maintained at low, nonpathogenic levels when a population is below the carrying capacity of the available habitat. As the number of animals increases to or exceeds the carrying capacity of the habitat, the percentage of animals infected and the severity of infection increase.⁵ The consequences of mismanaging populations of white-tailed deer, with the potential for increased disease and parasites, have been documented.³

As the deer population increased on public lands as a result of successful management efforts, biologists increased the numbers of antlerless permits and allowed baiting in an effort to increase the harvest. Large-scale baiting increased the physical contact and stresses, but also escalated as hunters baited on public lands to compete. The clubs and private landowners put out even bigger piles to attract and keep deer around their lands. This facilitated increased transmission, so more animals were infected, with disease lesions showing up even earlier. Tuberculosis could easily have gone undetected in a population within the carrying capacity of the area where only bucks are harvested, because the vast majority of antlered bucks taken are 1.5 to 2.5 years old in the fall hunt.

Animals with advanced lesions are able to infect other deer directly when in close contact, as at a bait or feeding station. Chewing and slobbering on large food items such as sugar beets could leave mycobacteria for the next deer to consume. A startled deer snorts and whistles, which may aerosolize organisms into the

environment, allowing inhalation and contamination of feed, water, and soil, again in these high-density areas. Studies have shown that *M. bovis* may be cultured from bait and feed as well as soil and water samples and may persist as a potential source of infection for at least 1 month under natural conditions.⁶

CONTINUED SURVEILLANCE

Tuberculosis in Privately Owned Cervids

After documentation of TB in free-ranging white-tailed deer, wildlife managers were convinced that there was a livestock source. Privately owned deer on game farms and ranches in Michigan are kept behind fences and are considered livestock. They are regulated by the state's DNR and Department of Agriculture.

Movement of captive deer was strictly regulated, and testing of all animals over 1 year of age was required on smaller, more intensively managed facilities (*game farms*). Deer could be darted or rounded up and run through a chute for whole-herd testing with the single cervical tuberculin (SCT) skin test, which must be read 3 days after the initial injection. Larger enclosures without handling facilities or with extensive areas of heavy cover (*game ranches*) could not round up all animals for whole-herd testing. Therefore a method of inspecting the carcasses of animals culled or harvested by hunters (*slaughter surveillance*) was developed.

The percentage of the herd requiring inspection was determined by U.S. Department of Agriculture (USDA) protocols. All testing, whether whole-herd or slaughter surveillance, had to be done by accredited veterinarians with special training and certification by USDA or the Michigan Department of Agriculture. If skin-tested animals were initially classified as suspect, follow-up comparative cervical tuberculin (CCT) skin testing was done by agency veterinarians. Culture confirmation was required on necropsy tissues for any suspicious findings on slaughter surveillance.

The rigorous testing at 96 captive-cervid facilities in the endemic TB area identified only one game ranch with TB in its herd. This herd has been intensively studied and documented because all animals were slaughtered.⁸

Because TB apparently had been in the wild deer longer than the infected game ranch had been in operation, it was thought that the likely source of infection was one or more of the 108 wild deer that had been initially enclosed when the fence had been completed in 1992. As noted, it is likely that spread of the disease was enhanced by the intensive captive

management with feeding and baiting for trophy hunters, just as similar conditions on the clubs increased the prevalence of TB in the free-ranging deer.¹¹

More than 12% of the depopulated herd cultured positive for *M. bovis*, many with no grossly visible lesions. The state has continued strict regulation of game ranching, which it considers a potentially risky practice, and requires that no wild deer may be enclosed by fences when a new facility is approved. The extensive snowfall of Michigan winters allows monitoring for a lack of deer tracks, thus confirming that no wild deer are enclosed. However, this does delay and add to the expense of opening new facilities, which presently are not allowed in the endemic area. The rigorous regulation and mandatory testing of livestock in the game farming and ranching industry in Michigan have eliminated most of the small “hobby farms” without handling facilities. Additionally, this has allowed the industry to deal with surveillance of other issues, such as testing for chronic wasting disease (CWD), which has not yet been found in Michigan deer in captivity or the wild.

Tuberculosis in Cattle Farms

Management strategies to control TB in wild deer involved regulating the practice of feeding and baiting. There was concern that deer that had depended on artificial food sources would now look for new food supplies and switch to cattle feed once artificial feeding was stopped.

Given that concern, required testing of livestock was initiated in the area and identified the first infected cattle herd in 1998. Statewide testing identified several more infected herds, and although all herds were in the area where the deer had TB, the whole state of Michigan lost its “tuberculosis accredited-free” status in 2000. Just as comingling of TB-infected cattle with deer had been the initial source of infection to the deer, now the infection was returning to cattle from the wild deer reservoir of infection.

Epidemiologic studies of environmental and farm practices on infected and uninfected farms showed that feeding cattle in wooded areas that provided more cover for deer increased the risk of infection, especially if a natural source of water (e.g., stream, pond) was present.⁹

Practices that kept cattle in and deer out of areas where food was stored or fed, such as using fences and feeding cattle in open areas without cover for deer, decreased the risk of cattle contracting *M. bovis* infection. Dairy cattle operations are at less risk than beef

operations because the latter are more likely to result in exposure to infected deer. Additionally, more beef cattle than dairy cattle are farmed in this area of inexpensive, poor-quality land.

A major risk factor to cattle was farm location in the area where deer had TB or near other farms where cattle had TB. This was expected, and after losing its tuberculosis accredited-free status, Michigan was able to achieve “split state” status in April 2004, allowing the area with TB to function in a more highly regulated status of “modified accredited,” with the rest of the state classified as “modified accredited advanced” in regard to official TB status. Michigan’s Upper Peninsula was designated “tuberculosis accredited-free” again in September 2005, resulting in the state being split into three different zones.

Tuberculosis in Michigan Elk (*Cervus elaphus*)

Michigan has a highly managed herd of elk in which surveillance has identified several individuals with *M. bovis*. The elk population is strictly managed to be kept within the carrying capacity of its habitat, which is in the same area as the deer TB problem. Even though elk are normally social and congregate more than deer, managers do not think the herd has a problem. These individual cases were believed to be the result of spillover infections from infected white-tailed deer and elk eating at the same bait station.

All harvested elk are examined and cultured. The elk are highly valued by Michigan hunters, who compete for all permits issued. It is believed that TB will not become a problem in the properly managed elk herd. Also, it is thought less likely that TB will be an issue as the problem is resolved in the deer population, but diligent, continued surveillance is warranted.

Tuberculosis in Other Species

Several species of wild carnivores, including black bear (*Ursus americanus*), bobcat (*Felis rufus*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and raccoon (*Procyon lotor*), as well as opossum (*Didelphis virginiana*), have been found to be infected with *M. bovis* during routine surveillance of hunter- and trapper-harvested animals. All these species appear to have contracted TB from ingesting infectious tissue from deer carcasses or gut piles and appear to be dead-end hosts with few lesions and little risk of spreading TB or becoming a reservoir of the disease.¹

A single domestic cat (*Felis catus*) was found in 2000 with disseminated *M. bovis* at a home in the middle of the area where all the other TB cases originated. Twenty cats from the same premise were rigorously tested, necropsied, and cultured in an epidemiologic investigation.¹⁰ The study found no other infected cats, although they all had been in close contact with the infected cat (>50 additional cats were on the owner's premises). Both the owner and the veterinarian who treated the cat before it died had negative skin tests for TB. However, the question of zoonotic risk remains when domestic house pets become infected.

Also, two human cases of *M. bovis* infection were typed as the same organism as all the other cases. One infection in 2004 was in an experienced bow hunter who cut his hand while dressing an infected deer.¹⁵ He was successfully treated for the local infection. This case drew media attention and decreased hunter interest in taking deer from the area. In the second case, in 2002, the organism was isolated from a pulmonary specimen obtained just before the death of a 74-year-old man whose wife had TB more than 40 years earlier.¹⁶ He contracted the infection from her, but did not develop clinical disease until other health issues compromised his immune system.

Deoxyribonucleic acid (DNA) fingerprinting, or restriction fragment length polymorphism (RFLP) analysis, of all positive species has confirmed that they all are the same strain of *M. bovis*. This suggests that this may be the same *M. bovis* strain that was common in Michigan cattle and humans at least 50 years ago, and that may have persisted in the white-tailed deer herd all this time.

MANAGEMENT EFFORTS

As hunter success diminishes in the area that has a complete ban on baiting, hunters will go to areas that allow limited baiting to be more successful. Managers have tried different unpopular baiting regulations and were unsuccessful at banning the practice statewide and, as noted earlier, even tried allowing limited baiting in the TB area in 2001 in an unsuccessful attempt to see if the harvest could be increased.¹² Unlimited antlerless permits, along with special and extended seasons, have done much to decrease the problem but have failed to lower the prevalence rate of infection below 1% to 2% for the last several years, with local "hot spots" of almost 5% in some locales.

Despite varied education efforts, hunters are reluctant to give up the long-standing tradition of "shooting deer over bait." If less than 2% of the deer are infected,

it further infuriates hunters to be told they need to kill even more deer. The low rate of infection has virtually no limiting effect on the deer population, and many deer hunters would be willing to deal with a population with some TB. They argue that their hunting is more important to the state's economy than cattle farming. Cattle farmers have seen a gradual decrease in the number of new and reinfected farms with the testing and slaughtering approach, but both dairy and beef herds continue to be found with TB.

The infectious reservoir is in the deer, and farmers think the solution is simple: eradicate the deer and TB will be gone as well. Both sides will need to compromise to solve this complex biologic and political ("biopolitical") problem. Some farmers will need to change where and how they feed their cattle if they want to avoid the problem. Hunters will have to accept fewer deer and learn to hunt without large bait stations. These compromises do not seem to be happening, and it may take some new tools to manage the problem and help, along with education, to change attitudes of stakeholders if the goal of eradication has any chance of succeeding.

FUTURE MANAGEMENT POSSIBILITIES

Progress has been made, but efforts must continue. Recent research efforts have been directed at developing a test-and-slaughter technique that might work on free-ranging deer. If wild deer may be live-trapped or darted (both methods probably need baiting to increase success) and tested on-site, uninfected deer could be released, with only infected deer being culled. This approach would be similar to the test-and-slaughter technique that has been successful with managing *M. bovis* in cattle and captive cervids. It has more appeal to hunters because it would save healthy deer from a population that is already low. This is likely to be more "politically" acceptable than removing all the deer, as presently done.

In 2003 a protocol was developed to evaluate the feasibility of using the gamma-interferon assay in a research project in which more than 100 deer were live-trapped, tested, fitted with radio transmitters, and released.¹² If a blood test or culture was positive, the animal could be relocated and culled. This was a labor-intensive and expensive project, but it was reasonably well accepted by hunters and landowners and did show that the protocol was possible.

There were technical problems with the blood test, and several other diagnostic blood tests were evaluated

during the 2004 season, with only limited success. This research is expensive, but protocols for capture and sampling have been successful, and when useful diagnostic tests become available, this “trap, test, and release” protocol could be used as a management tool in areas that remain as hot spots of higher rates of infection.

If an effective TB vaccine is developed, uninfected deer could be injected at the time of capture. Some of the new developments using recombinant subunit vaccines to develop antibodies to *Mycobacterium* spp. hold promise. An oral vaccine would be even better and extremely applicable at the herd management level because the delivery system (baiting and feeding) is already in place. Even a vaccine that provided some limited immunity would be a useful tool to augment the strategy of drastically decreasing numbers of deer and changing hunters’ attitudes over a ban on congregating deer over bait.

Tuberculosis is likely to persist for some time in Michigan deer unless there is new technology developed for diagnostic testing and vaccination.¹² Even then, hunters will need to compromise and be willing to accept fewer deer that are more challenging to hunt without large bait stations. Farmers will have to compromise as well and make greater efforts to store livestock feed and to feed their livestock in ways that minimize access by wild deer, which presently remain the infectious reservoir.

Education of both hunters and farmers to facilitate continued cooperation and compromise to accept new management tools, along with giving traditional tools a chance to eradicate TB, is essential for any management strategy to succeed. The biopolitics of this complex problem are similar to the problem of brucellosis in elk and bison on the feeding grounds in the Greater Yellowstone area of the United States.⁷

The goal of complete eradication of TB from Michigan’s deer herd is currently unattainable, but it is a worthwhile goal to try to achieve. Tuberculosis may at least be reduced to the level where it was before its detection. With proper management of both livestock and wild deer, it should be possible to keep TB at such a low level that with some help by a natural event, such as a particularly severe winter, it eventually will be “gone” from the healthy deer herd, or at least below detectable limits, and “eradicated” by some epidemiologists’ statistically significant definition.

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Chronic Wasting Disease of Cervid Species

MICHAEL W. MILLER

Chronic wasting disease (CWD), a contagious prion disease of several cervid species, has emerged from obscurity to become what some consider one of the most important wildlife health problems in North America. Although the long-term implications and relative importance of CWD remain to be determined, the detection and apparent extent of CWD in both free-ranging and captive cervid populations already have had appreciable impacts on both wildlife management and the captive cervid industry⁴⁴⁻⁴⁶ (Figure 53-1).

The 1990s and early 2000s were largely a period of discovery with respect to CWD. The extent and potential severity of CWD epidemics began to be appreciated in free-ranging and captive populations. Improved diagnostic and surveillance tools were developed, and insights were gained into its epidemiology and potential host range. Armed with existing tools and knowledge, animal health and wildlife management professionals are now challenged to craft practical and effective strategies for detection, control, and prevention. At the same time, wildlife professionals recognize that knowledge and tools will continue to improve with better understanding of CWD and other prion diseases in coming years.

Chronic wasting disease presents a diagnostic and epidemiologic challenge for veterinarians working with captive and free-ranging cervids because clinical signs are subtle and nonspecific throughout much of the disease course, antemortem diagnostic tools are limited, and information on relevant history and risk factors often is incomplete. In light of recent trends, however, practitioners of captive or free-ranging cervid medicine in North America should consider the potential for encountering CWD when developing herd health monitoring and management programs.

ETIOLOGY

Chronic wasting disease is one of a group of unconventional diseases originally termed “transmissible spongiform encephalopathies” (TSEs) and is one of three TSEs that occur in domestic ruminants in North America; the other two are scrapie of sheep and goats and bovine spongiform encephalopathy (BSE) of cattle. The TSEs appear to be caused by proteinaceous infectious agents (*prions*),³³ although a viral or virinal etiology has not been completely disproved. As with other prion diseases, the CWD agent (prion or otherwise) has not been isolated or fully characterized. A single strain of CWD prion has been recognized thus far, but data from several studies suggest the possibility of CWD strain variation.^{3,34,35} The relatively wide natural host range (four species in three different genera) and polymorphisms in the prion gene of each host species provide a plausible biologic mechanism for such variation in cervid-associated prion strains to arise.

The origin of CWD is not known, but three possible sources have been hypothesized.^{44,45,50} First, CWD may be a new or a reemerging disease of cervids; its relatively limited global distribution and poor infectivity in bovids support this hypothesis. Second, CWD may be the result of scrapie infection in cervids; the many similarities to scrapie pathogenesis and epidemiology and lack of cohesive epidemiologic explanations for all the CWD foci detected in North America support this hypothesis. The third, more remote possibility is that CWD arose from an as-yet-unrecognized prion strain originating in another domestic or free-ranging species. The relationship between CWD and scrapie and the potential for scrapie exposure to initiate new CWD foci in North America and elsewhere have not been

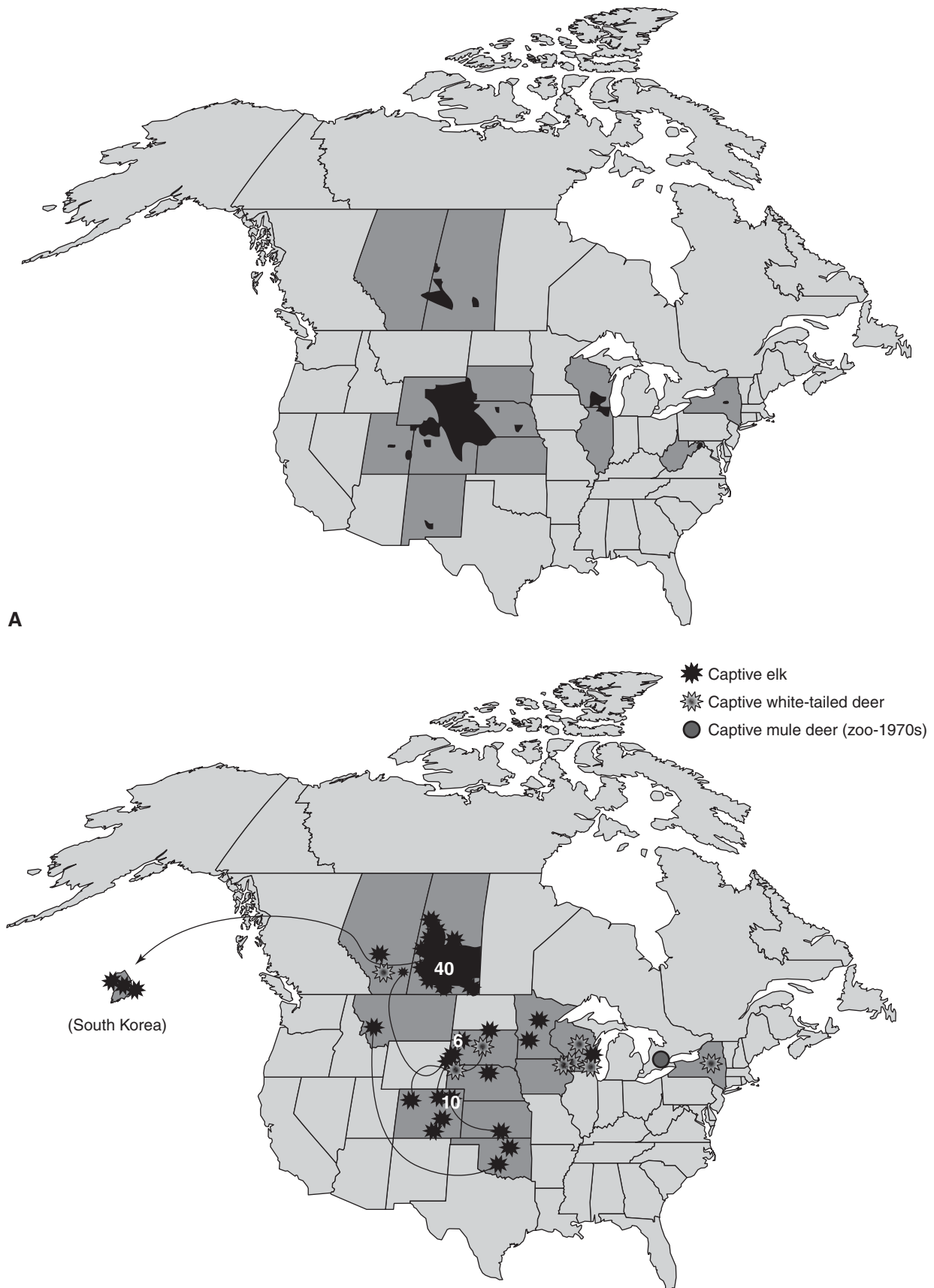


Fig 53-1 Approximate known distribution of chronic wasting disease (CWD) in **A**, free-ranging cervids, and **B**, captive cervids. Locations are approximate, and data are compiled from a variety of published and unpublished sources. Most infected captive facilities were depopulated once CWD was detected.

defined. Although some uncertainty exists about the relationship between CWD and scrapie prion strains, laboratory and epidemiologic data have shown more clearly that the CWD strain is different from the prion strain that causes BSE.^{4,34,43}

EPIDEMIOLOGY

Chronic wasting disease occurs naturally in four species in three genera, all North American members of the family Cervidae. Infections in mule deer (*Odocoileus hemionus*) and white-tailed deer (*O. virginianus*) are more common than in wapiti (*Cervus elaphus nelsoni*)^{28,45}; a single natural case has been reported recently in moose (*Alces alces*).²⁰ There appear to be molecular barriers limiting the potential natural host range of CWD.³⁵ The notion of a limited natural host range for CWD is consistent with its absence in other free-ranging or domestic North American ruminant species with opportunities for natural exposure.^{10,46} As with other prion diseases, however, CWD has been transmitted to a variety of species by experimental intracerebral inoculation.^{44,45,50}

Chronic wasting disease is contagious among susceptible species, but knowledge about the exact mechanism(s) of natural transmission remains incomplete. Shedding of agent in feces with fecal-oral transmission seems the most plausible route of CWD transmission, but other possible mechanisms cannot be discounted.^{23,26,38,45,49} Regardless of the precise mechanism, direct or indirect horizontal transmission apparently sustains epidemics. Maternal transmission, if it occurs, is of limited importance.²⁶ Live, infected animals likely are the main sources of new infections and geographic spread.* Environments contaminated with excreta or decomposed carcasses may harbor some infectivity for years.²³ No evidence has emerged suggesting that CWD is associated with a food-borne pathogen, as is the case with BSE. Deer and wapiti held in affected game farms have not been shown to have consumed rendered ruminant proteins. Similarly, free-ranging deer and wapiti feed on natural forage and have not had known contact with rendered feeds or byproducts in places where CWD epidemics have occurred.

Genetics appear to influence CWD susceptibility and pathogenesis.⁴⁴ For the three main natural host species, one or more substitution polymorphisms have been identified in the protein-coding region of the native prion gene in wapiti at codon 132 (methionine [M] or

leucine [L]),³⁰ in mule deer at codons 20 (aspartate [D] or glycine [G], removed during processing) and 225 (serine [S] or phenylalanine [F]),² and in white-tailed deer at codons 95 (glutamine [Q] or histidine [H]), 96 (G or S), and 116 (alanine [A] or G).³¹ Deer and wapiti of all recognized prion protein (PrP) genotypes may be infected with CWD; however, individuals of the less common genotypes in each host species tend to be underrepresented among infected conspecifics, and the onset of clinical disease in such individuals may be delayed.^{12,15,16,30,31}

Cases of CWD may occur at any time of year. In the wild, however, these tend to be observed more often in the fall and winter^{28,41}; a similar pattern also occurs in captive cervids. Among mule deer, infection rates are higher in males and older individuals, suggesting differential exposure risks between genders and across age classes^{22,28}; similar patterns would be expected in the other host species.

Although surveillance for CWD began in some areas in the early 1980s, most of the effort directed toward determining its distribution and prevalence in free-ranging and captive cervids in North America and elsewhere has occurred since 2000.^{28,41,45,47,50} Detecting CWD foci in free-ranging populations is challenging because disease distribution appears to be patchy and sampling is rarely uniform.^{28,37} Overall prevalence of CWD infection in endemic areas may reach 1% to 5% or higher in free-ranging populations.^{17,28} The perception that CWD has “spread” dramatically since 2000 seems more likely an artifact of the increase in surveillance activities undertaken in recent years rather than a true expansion in the number of new foci over this period.

Under captive conditions, CWD prevalence may be even higher than observed among cervids in the wild. In captive mule deer and white-tailed deer, entire cohorts have become infected and succumbed to CWD over the course of several years.^{23,26,48} High prevalence also has been reported in captive wapiti, but this pattern is not as consistent as seen in captive deer.^{18,24,32,49} Factors contributing to the remarkably high prevalence sometimes seen in captive deer and wapiti remain to be determined, although intensive environmental contamination and repeated exposure to infectious materials seem likely contributors.

CLINICAL SIGNS AND COURSE

The image of a drooling, stumbling, emaciated deer or wapiti has been represented and repeated so often as the “classic” presentation for clinical CWD over the

*References 6, 17-19, 39, 45, 46, 50.

years that many clinicians, wildlife managers, and cervid caretakers might not recognize a more typical clinical case if presented with one (Figure 53-2, A). It follows that failure to recognize and properly investigate suspect CWD cases may be partially responsible for delayed detection of foci in both captive and free-ranging cervids and for gaps in epidemiologic investigations of its occurrence and spread.

Both the clinical course and the clinical presentation of CWD in deer and wapiti may vary widely, as with many other diseases. It is true that the most recognizable signs of end-stage CWD in adult cervids are

behavioral alterations and loss of body condition. As an added complication to clinical diagnosis, consistent clinical signs occur relatively late in the overall disease course, and other health problems or interceding causes of death (e.g., accidents, predation) often confound clinical presentations.

Clinical signs seen in CWD cases are nonspecific and may include behavioral changes; ataxia; head tremor; hyperexcitability; hyperesthesia; piloerection; intermittent tremors (primarily in wapiti); dilated, spastic, or flaccid esophagus; abnormal movements of the tongue; sialorrhea; odontoprisis; dysphagia and swallowing difficulties; loss of body condition; polydipsia; and polyuria. Some of these signs may be most obvious during or after handling, anesthesia, or other stressful situations. Pruritus, sometimes seen in scrapie, has never been reported in CWD cases.

In captive animals the earliest signs of CWD are subtle changes in individual behavior, usually detected by caretakers most familiar with the individual animal. These typically include changes in the way the animal interacts with caretakers or other animals in the herd and in responses to unusual stimuli; for example, previously intractable animals may appear to become tame. Repetitive behaviors or periods of somnolence are seen less frequently. Early signs are often so subtle that they may be inapparent to those not familiar with the affected individual, even when such observers are clinicians with considerable experience in recognizing CWD. Signs become more consistent and recognizable as disease progresses.

Affected animals eventually lose weight even though they continue to eat, but weight loss may be episodic.²⁴ In individuals that survive to the terminal stages of CWD, body condition will decline appreciably and irreversibly. Clinical signs tend to be more difficult to recognize and more rarely reported in free-ranging cervids, perhaps because they may mimic normal seasonal variation in behavior and condition, and because obviously affected individuals may not survive long enough in the wild to show signs consistently (Figure 53-2, B).

In all cases to date, affected animals did not recover from clinical CWD.^{44,45,50} As with its signs, the clinical course of CWD is variable and may last from a few weeks to perhaps a year or more. In part, the apparent duration of clinical disease depends on the astuteness of observers in recognizing subtle early clinical signs. In deer the clinical course is sometimes very short, lasting only a few days, and infected animals have died acutely without displaying typical clinical signs.²³ "Sudden deaths" following handling also have been reported in some situations, as have unusual mortalities

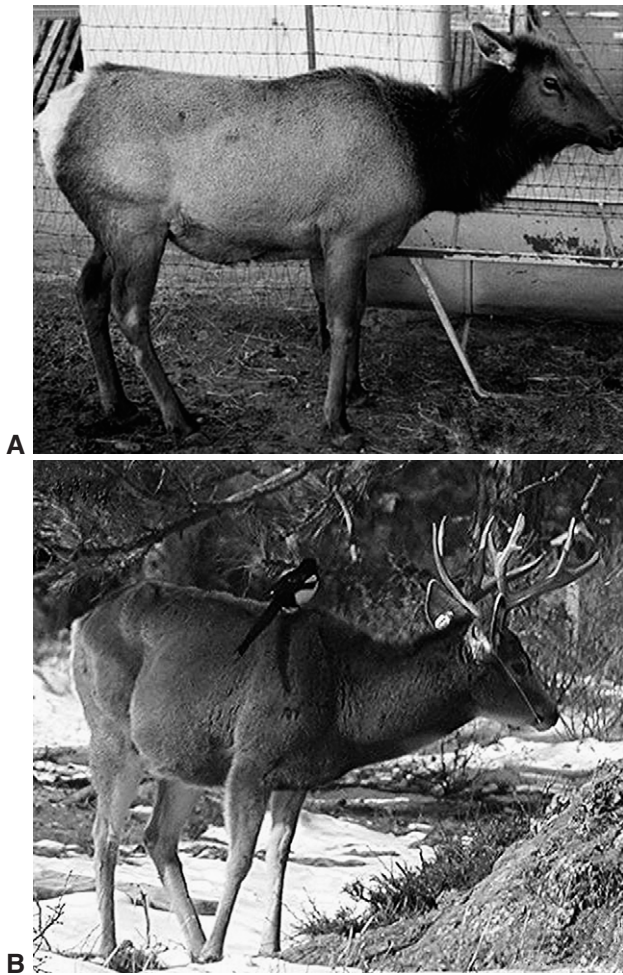


Fig 53-2 Clinical chronic wasting disease (CWD) in **A**, captive female wapiti, and **B**, free-ranging male mule deer. The female wapiti had been showing subtle signs of CWD, mostly changes in response to handling and interaction with herd mates, for more than 6 months before the photo was taken. The wapiti was euthanized about 3 months later after signs progressed, although still not to classic end-stage CWD. The male mule deer showed signs that included cachexia, piloerection, diminished alertness, and vacant facial expression (all evident in photo), and mild ataxia also was appreciable when the deer moved. (See Color Plate 53-2.) (A courtesy M.W. Miller; B courtesy S.W. Miller; reprinted with permission, all rights reserved.)

(e.g., an animal getting its head caught under a feed trough or entangled in a low fence). The PrP genotype also may influence the overall length of the disease course in both deer and wapiti.^{12,15}

Clinical CWD in both deer and wapiti is preceded by a relatively long incubation period. Little is known about the average or maximum length of the incubation period for natural infections, but because end-stage clinical CWD has been reported in deer and wapiti less than 2 years of age, a minimum of 1 to 1.5 years seems likely. Such estimates are consistent with observations of incubation periods after oral experimental infections.^{44,45} Clinical CWD has been manifested in individuals as old as 15 years, but in such cases of older onset, the timing of exposure has not been determined.^{45,50} The onset and severity of clinical CWD appear to coincide generally with the timing and progression of abnormal prion accumulation and spongiform degeneration in the brain and spinal cord of infected individuals.

There are many potential differential diagnoses for clinical CWD. Behavioral alterations and central nervous system signs, as well as weight loss, may have a variety of other causes in cervids, including paraplasmogonylosis, bacterial or viral meningitis or meningoencephalitis, trauma, intoxication, and brain abscesses. Emaciation of cervids may also occur for many reasons besides CWD. Consequently, a wide variety of diseases and conditions that lead to loss of body condition should be considered as differential diagnoses. Some possibilities include malnutrition or starvation, dental attrition, foreign body obstruction, and musculoskeletal problems preventing normal foraging behavior. Some infectious diseases leading to poor body condition include parasitism, paratuberculosis, tuberculosis, and chronic bronchopneumonia. In Colorado, three mule deer submitted as CWD “suspects” were eventually diagnosed as cases of plague.

In addition, changes in body condition attributable to disease need to be distinguished from the seasonal fluctuations in condition that occur naturally in both free-ranging and captive cervids. Drooling and dysphagia may be caused by many of the same conditions that lead to loss of body condition; in addition, rabies always should be included in the differential diagnosis. Esophageal reflux and difficulties in swallowing caused by CWD may lead to illness or death due to aspiration pneumonia, which occurs with some frequency among CWD cases, particularly in captive cervids. Consequently, CWD always should be suspected and ruled out when unusual cases of pneumonia are encountered in captive North American deer or wapiti or closely related species, especially when

poor body condition or behavioral changes are also apparent.

DIAGNOSIS

Because the clinical signs of CWD are neither consistent nor diagnostic, its diagnosis must be confirmed by laboratory examination; a recent review⁴⁴ provides more extensive discussion of CWD diagnostics. Briefly, CWD may be diagnosed through examination of the brain for spongiform lesions^{48,49,51} or through examination of brain or lymphoid tissues by immunohistochemistry (IHC) for accumulation of CWD-associated prion protein (PrP^{CWD}).^{25,28,32,40,41} The histopathology of CWD has been described extensively.^{43,48,49,51} The parasympathetic vagal nucleus in the dorsal portion of the medulla oblongata at the obex is the most important site to examine for diagnosing CWD.^{44,51} This nucleus shows consistent, relatively early involvement after CWD infection in all four known susceptible species. Optimally, the obex should be preserved in 10% buffered formalin, and remaining portions of brainstem should be frozen for prospective use in confirmation or strain typing.

Detecting accumulation of PrP^{CWD} in brain and lymphoid tissues using IHC has largely replaced histopathology in diagnosing CWD.^{25,28} Of the several monoclonal antibodies (MAbs) evaluated for CWD diagnosis, MAb 99/97.6.1²⁹ has proved most reliable.^{25,40,42} Demonstration of PrP^{CWD} in the parasympathetic vagal nucleus at the obex of the medulla oblongata is both sensitive and specific for CWD diagnosis in deer and wapiti.^{25,28,40} Because PrP^{CWD} accumulates in lymphoid tissues of deer and wapiti early in infection,^{38,44} lymphoid tissue IHC also has been used to diagnose CWD. Tonsils or retropharyngeal lymph nodes have proved particularly reliable for diagnosing CWD in deer using IHC, thus providing a foundation for both antemortem and preclinical postmortem diagnostic applications.^{25,52} Both tonsils and retropharyngeal lymph nodes are readily collected from heads of harvested and slaughtered animals and may be examined alone or in combination with brainstem to enhance diagnosis.

Enzyme-linked immunosorbent assays (ELISAs) originally developed for BSE testing also have been used in CWD diagnostics, primarily as screening tests in harvest surveys in which large numbers of samples are submitted for testing.¹⁴ Although several ELISAs appear to offer good sensitivity and specificity as screening tests, suspected cases identified by ELISA should be confirmed by IHC before a diagnosis of

CWD is finalized in either captive or free-ranging cervids.

TREATMENT

No effective therapeutic approaches have been identified for treating or curing CWD infections in individual cervids. Supportive therapy may prolong the lives of animals with clinical CWD for a short time, but doing so seems inadvisable in the context of herd health management. Although several compounds show some promise for treating clinical prion disease or preventing prion infection,^{5,36} their application to either captive or free-ranging cervids thus far appears impractical given limitations in technology. Similarly, strategies for vaccination or immunotherapy to prevent or treat prion infections also may hold promise, but none is available for field use.^{1,8}

Early detection and aggressive removal of infected individuals may be an effective approach for controlling (but perhaps not completely eliminating) CWD in captive wapiti populations.^{21,24} The effectiveness of such an approach likely will depend on (1) how quickly new cases may be detected and removed from a herd, (2) the extent of proper husbandry to minimize buildup of and exposure to potentially infectious excrement and carcass remains, and (3) the cumulative number of cases that have occurred in the facility. Examination of tonsil⁵² or rectal mucosa^{9,21} biopsies with IHC could be used to augment regular clinical evaluations in screening for early cases.

In most cases, captive wapiti or deer on infected game farms have been depopulated in an attempt to eliminate infection. Attempts to eliminate CWD from wildlife research facilities in Colorado and Wyoming through depopulation and decontamination were not successful, but the reasons for failures were unclear.^{24,50} Recurrence of CWD in these facilities could have been caused by residual environmental contamination or by reintroduction of infected animals into the facilities, although the former seemed most likely. More extensive recommendations for such approaches have been developed in recent years,¹⁸ but the long-term efficacy of such approaches in completely eliminating CWD remains to be evaluated.

CONTROL

Control of CWD in free-ranging cervids appears to be even more problematic.^{11,47} Attempts to either control or eliminate CWD in free-ranging populations through

broad and local population reductions and selective removal of infected animals have been undertaken relatively recently, and it is too early to assess the effectiveness of these attempts.^{47,53}

Regardless of the approach for addressing infected herds, the ultimate success of efforts to control CWD appears dependent on early detection of new foci and sources of infection. To this end, mortalities of adult cervids in captivity should be examined for evidence of CWD not only in North America, but wherever prion diseases occur in domestic or wild ruminant species. Recognition of CWD in the commercial cervid industry in North America has prompted development and implementation of herd monitoring and certification programs. Surveys of free-ranging populations for evidence of CWD infection also are underway in many parts of North America and elsewhere.

Several approaches have been used separately and in combination to detect new foci of CWD. Common methods include collecting and testing "suspect" deer, wapiti, or moose showing clinical signs compatible with CWD; sampling harvested cervids during annual hunting seasons; and sampling vehicle-killed cervids.^{20,28,37} Strategies focusing on symptomatic or vehicle-killed animals tend to bias sampling toward detecting infected individuals and thus may be better for detecting new CWD foci. Live-animal testing with tonsil biopsy also has been used to augment CWD surveillance in urban and exurban areas.^{7,52,53}

PREVENTION

Preventive measures intended to reduce opportunities for further spreading and establishing new foci of CWD include (1) surveillance programs for captive and free-ranging populations, (2) regulations preventing translocation or movements in commerce of live animals and carcass parts from endemic regions or directly neighboring wild populations to other areas, and (3) regulations prohibiting release of animals from captive herds into free-ranging populations. Additionally, it is recommended to double-fence established game farms in the CWD-endemic areas and to discourage establishing new captive facilities in such areas.

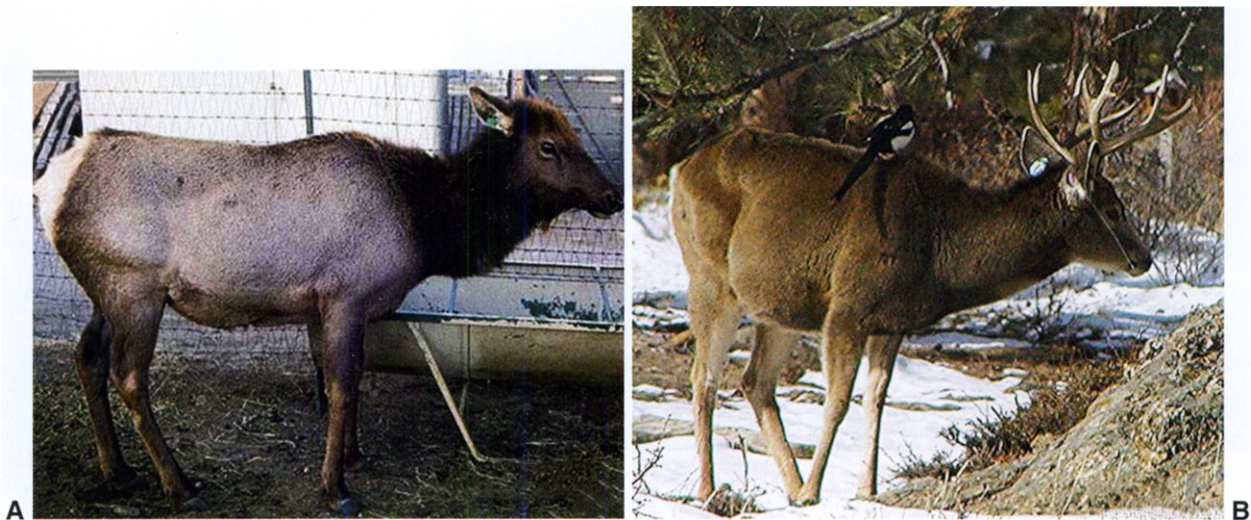
Without detailed knowledge about the origin(s) and relative importance of various transmission routes of CWD, it is difficult to determine what risk factors are associated with its geographic spread and establishment among captive and free-ranging cervid populations. Direct contact with deer or wapiti with CWD or from known infected or exposed herds should be considered a risk factor for susceptible animals. Also,

because of the possibility of environmental contamination with the CWD agent, housing cervids on pastures or in facilities or regions that have previously held animals with CWD also should be considered a possible risk. Scrapie exposure also should be seen as a potential risk factor, particularly in cases in which other, more likely risk factors cannot be identified or are relatively implausible.

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Color Plate 53-2 Clinical chronic wasting disease (CWD) in **A**, captive female wapiti, and **B**, free-ranging male mule deer. The female wapiti had been showing subtle signs of CWD, mostly changes in response to handling and interaction with herd mates, for more than 6 months before the photo was taken. The wapiti was euthanized about 3 months later after signs progressed, although still not to classic end-stage CWD. The male mule deer showed signs that included cachexia, piloerection, diminished alertness, and vacant facial expression (all evident in photo), and mild ataxia also was appreciable when the deer moved. (For text mention, see Chapter 53, p. 433.) (A courtesy M.W. Miller; B courtesy S.W. Miller; reprinted with permission, all rights reserved.)

Heartwater (*Ehrlichia ruminantium*)

SHARON L. DEEM

DISEASE DEFINITION

Heartwater is a noncontagious tick-borne disease of domestic ruminants and wildlife. Thought to be a wildlife-adapted disease, heartwater was first recorded in livestock after it spilled over into a domestic sheep in South Africa in 1838.²⁸ Heartwater is one of the most devastating livestock (cattle, sheep, goat) diseases in sub-Saharan Africa, with mortality rates up to 80% in naive domestic livestock. In addition to sub-Saharan Africa, heartwater is now present in Madagascar, various small islands in the Indian Ocean and Atlantic Ocean, and islands in the eastern Caribbean Sea.

The causative agent, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*), is an obligate intracellular rickettsial agent, classified as a member of the Ehrlichiae tribe in the family Rickettsiaceae, order Rickettsiales.¹⁴ Three morphologic forms of the organism are recognized; elementary (electron dense), intermediate, and reticulate bodies.²⁹ Cells are initially infected by elementary bodies, through a process resembling phagocytosis. Thereafter, the organisms divide by binary fission inside intracytoplasmic vacuoles to form large colonies of reticulate bodies. The reticulate bodies then develop into smaller intermediate bodies with an electron-dense core, before condensing further to form elementary bodies. Infected cells rupture and release elementary bodies into the bloodstream to infect other cells and, presumably, ticks feeding on the host.²⁹ Protective immunity to heartwater in the vertebrate host is considered to be primarily cell mediated, although *E. ruminantium* antibodies are detected in serum and colostrum and most likely play a role in immunity.

Until the mid-1980s, when the in vitro cultivation of *E. ruminantium* was achieved, little was known about the epidemiology of heartwater because of the lack of antemortem diagnostic capabilities. Even today, diagnosis of heartwater remains difficult in both domestic and wildlife species. Additionally, the lack of a safe

and effective vaccine has limited our ability to control this disease.

There is growing concern that *Amblyomma* spp. tick vectors and the disease-causing rickettsial agent may be introduced into livestock and wildlife currently living in heartwater-free regions.¹² Recent epidemiologic discoveries (e.g., wildlife and domestic carriers and vertical transmission), as well as anthropogenic factors (e.g., animal trade), support the likely introduction of *E. ruminantium* to regions presently free of the disease. *Ehrlichia ruminantium* is an Office International des Epizooties (OIE) list B reportable disease.

EPIDEMIOLOGY

The only known vectors capable of transmitting *E. ruminantium* are several species of ticks in the *Amblyomma* genus. The African ticks *A. variegatum* and *A. hebraeum* are the two most important vectors on a global scale.³⁴ Three of the 13 *Amblyomma* spp. known to transmit *E. ruminantium* naturally or experimentally, *A. maculatum*, *A. cajennense*, and *A. dissimile*, are American ticks.^{5,16} Of more concern for the American mainland is *A. variegatum*, the most efficient vector of *E. ruminantium*, which was introduced into Guadeloupe Island in the early 1800s and subsequently became established on a number of islands in the Caribbean⁴ and has, on occasion, been imported into the United States.

Amblyomma spp. ticks have been documented to infest more than 100 species within the Aves, Mammalia, and Reptilia vertebrate classes.¹⁰ The large host range of *Amblyomma* spp. ticks, the ability of both transstadial and intrastadial transmission of *E. ruminantium* within the tick, and the three-host life cycle of *Amblyomma* spp. ticks allow an individual tick to spread the disease to more than one host. *Amblyomma* spp. ticks also have qualities, such as aggregation-attachment pheromone (AAP) used in host location

and selection,²¹ continual long-term feeding of males on hosts,¹ and the ability to maintain their infectivity after infecting a host,¹ that contribute to the attainment of endemic stability in many parts of sub-Saharan Africa.

Five species of domestic animals—cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*), water buffalo (*Bubalus bubalis*), and domestic ferret (*Mustela putorius furo*)—are known to be susceptible to infection with *E. ruminantium*. Cattle, sheep, and goat are the most significant domestic species associated with the epidemiology of the disease. Wildlife ruminants have often been considered carriers, although clinical cases of heartwater do occur in wildlife species. The literature contains many reports of wildlife species that have experienced subclinical and clinical infections. These reports, reviewed in Deem¹² and Peter et al.,²⁷ include African buffalo (*Syncerus caffer*), African elephant (*Loxodonta africana*), Barbary sheep (*Ammotragus lervia*), black rhinoceros (*Diceros bicornis*), black wildebeest (*Connochaetes gnou*), blesbok (*Damaliscus pygargus*), blue wildebeest (*Connochaetes taurinus*), chital (*Axis axis*), crowned guinea-fowl (*Numida meleagris*), eland (*Taurotragus oryx*), fallow deer (*Dama dama*), four-striped grass mouse (*Rhabdomys pumilio*), giraffe (*Giraffa camelopardalis*), greater kudu (*Tragelaphus strepsiceros*), Himalayan tahr (*Hemitragus jemlahicus*),

impala (*Aepyceros melampus*), Indian spotted deer (*Axis axis*), lechwe (*Kobus leche kafuensis*), leopard tortoise (*Geochelone pardalis*), rusa deer (*Cervus timorensis*), sable antelope (*Hippotragus niger*), scrub hare (*Lepus saxatilis*), sitatunga (*Tragelaphus spekii*), southern multimammate mouse (*Mastomys coucha*), steenbok (*Raphicercus campestris*), timor deer (*Cervus timorensis*), tsessebe (*Damaliscus lunatus*), waterbuck (*Kobus ellipsiprymnus*), white rhinoceros (*Ceratotherium simum*), and white-tailed deer (*Odocoileus virginianus*). However, because of poor diagnostic capabilities and problems with cross-reactions, some of these infections have been questioned as to their authenticity. For example, recent experimental data suggest that both leopard tortoises and crowned guinea-fowl cannot be infected with *E. ruminantium*.²⁶ Peter et al.²⁷ summarize those wildlife hosts in which natural and experimental heartwater infections have been confirmed. These animals include 12 African ruminants, three non-African ruminants, and two African rodents (Table 54-1). However, it is probable that other wildlife species can be infected with *E. ruminantium*, and surveillance must be maintained to diagnose both carrier and clinically infected animals.

A long-term carrier state, first demonstrated in domestic cattle, sheep, and African buffalo, can be maintained for 246, 223, and 161 days, respectively.²

-Table 54-1

Nondomestic Species Confirmed Susceptible to *Ehrlichia ruminantium* Infection

Experimentally Infected	Naturally Infected
African ruminants	
African buffalo (<i>Syncerus caffer</i>)	Lechwe (<i>Kobus leche kafuensis</i>)
Black wildebeest (<i>Connochaetes gnou</i>)	Sitatunga (<i>Tragelaphus spekii</i>)
Blesbok (<i>Damaliscus pygargus</i>)	Springbok (<i>Antidorcas marsupialis</i>)
Blue wildebeest (<i>Connochaetes taurinus</i>)	Steenbok (<i>Raphicercus campestris</i>)
Eland (<i>Taurotragus oryx</i>)	
Giraffe (<i>Giraffa camelopardalis</i>)	
Greater kudu (<i>Tragelaphus strepsiceros</i>)	
Sable antelope (<i>Hippotragus niger</i>)	
Non-African ruminants	
White-tailed deer (<i>Odocoileus virginianus</i>)	Chital (<i>Axis axis</i>)
	Timor deer (<i>Cervus timorensis</i>)
African rodents	
Four-striped grass mouse (<i>Rhabdomys pumilio</i>)	
Southern multimammate mouse (<i>Mastomys coucha</i>)	

Reviewed in Peter TF, Burridge MJ, Mahan SM: *Trends Parasitol* 18(5):214-218, 2002, with references available of original citation for each species.

Subsequently, seven other experimentally infected African ruminant species (black wildebeest, blesbok, blue wildebeest, eland, giraffe, greater kudu, and sable antelope) have demonstrated subclinical carrier status.^{23,24}

In addition to the discoveries of new *Amblyomma* spp. vectors, high *E. ruminantium* infection rates in *Amblyomma* spp. ticks,²⁰ and domestic and wildlife hosts capable of being long-term carriers of *E. ruminantium*, vertical transmission in cattle and the influence of colostrum in calfhood immunity have been demonstrated.¹³ All these discoveries—high tick infection rates, long-term carrier status of vertebrate hosts, vertical transmission of the agent, and influence of maternal immunity on neonatal immunity—explain the finding of endemic stability, now considered the epidemiologic state of heartwater in much of sub-Saharan Africa, where the *Amblyomma* spp. vectors exist and indigenous livestock and wildlife are present.^{13,21,22}

DIAGNOSIS

One of the greatest challenges to heartwater research and control has been the lack of a reliable and easy antemortem diagnostic test. *Ehrlichia ruminantium* has a predilection for endothelial cells, and thus organisms are not detected on blood smears because of a limited rickettsemia. In fact, the only definitive antemortem test currently remains brain biopsy of infected animals with organisms detected in brain and intima vascular endothelial cells.³¹ Brain biopsies, used in conjunction with xenodiagnostics (tick transmission studies), are often employed with microscopic identification of the organism in the brain of a susceptible small ruminant. *Ehrlichia ruminantium* is a small, round organism (0.2–0.5 μm) that is gram negative and stains reddish to purple with Giemsa stain in biopsy samples.

In domestic ruminants and susceptible wildlife species, heartwater ranges from subclinical infection (carrier state) to a peracute, fatal disease. The incubation period varies based on species affected, route of infection, strain of *E. ruminantium*, and type and amount of inoculum. Clinical signs range from mild transient fever in subclinical cases, gastrointestinal and neurologic signs in acute cases, to death without premonitory signs in peracute cases.³³ The acute form, characterized by rapid onset of fever, tachypnea, inappetence, and neurologic signs (hyperesthesia, high-stepping or unsteady gait, twitching eyelids, chewing, abnormal tongue movement, individual muscle tremors), is the most common presentation in suscep-

tible naive hosts and often results in death.^{19,33} In domestic cattle, profuse, fetid, hemorrhagic diarrhea is typically reported. Because of the diverse clinical signs, differential diagnoses for heartwater are numerous and include any disease that causes gastrointestinal and neurologic signs or peracute death in domestic and non-domestic ruminants.

Clinical pathologic changes are often variable. The most frequently recognized alterations include progressive anemia, marked decline in thrombocytes, fluctuations in total and differential white cell counts, increased total bilirubin, and a decrease in total serum proteins.³²

A number of serologic and molecular diagnostic techniques have been developed in the last 20 years. However, poor sensitivity and specificity continue to limit the validity of most of these tests. Additionally, none of these tests has been validated in wild animals. A major limiting aspect to serologic assays is the cross-reactions with other *Ehrlichia* spp. Currently, serologic tests include the indirect fluorescent antibody test (IFAT), Western blot assay, a variety of enzyme-linked immunosorbent assays (ELISAs), including the ELISA, competitive ELISA (cELISA), polyclonal competitive ELISA (PC-ELISA), and MAP1-B ELISA. Although serologic tests continue to improve in sensitivity and specificity, limited validation in wildlife species, cross-reactions with *Ehrlichia* spp., and known seronegative responses in positive cattle³⁰ make serodiagnostics a poor choice for screening of individual animals.

Deoxyribonucleic acid (DNA) probes and polymerase chain reaction (PCR) assays have greatly advanced the detection of *E. ruminantium* in infected animals and ticks. The pCS20 PCR assay is presently the most reliable and best characterized test for *E. ruminantium* infection.^{17,25} The lack of cross-reactions with closely related organisms, such as *E. chaffeensis* and *E. canis*, increases the value of this test.

The pathophysiology of heartwater is poorly understood. *Ehrlichia ruminantium* parasitizes vascular endothelial cells, neutrophils, and macrophages of mammalian hosts. Macroscopic pathologic lesions and histologic findings are related to an increase in capillary permeability, which leads to the excess effusion of fluid into tissues and the body cavities. Postmortem findings associated with *E. ruminantium* include hydrothorax, pulmonary edema, ascites, hydropericardium (thus the name “heartwater”), cerebral edema, edema of the lymph nodes, and splenomegaly.¹⁹ However, these gross pathologic findings are variable and unreliable for making a diagnosis. Definitive postmortem diagnosis can be obtained by brain smears showing the organisms in endothelial cells that stain positive

with Giemsa stain. In addition to the brain, organisms may be identified by light microscopy in kidney, lung, and heart tissue.

TREATMENT AND CONTROL

Treatment of clinically ill animals is of limited value. Mortality is quite high once clinical signs develop, regardless of treatment. I have not successfully treated a clinical case after the onset of neurologic or gastrointestinal signs, but have successfully treated domestic ruminants by administering antibiotics at the start of a febrile response. Sulfonamides and tetracyclines are the drugs of choice. In domestic ruminants, oxytetracycline (6-10 mg/kg intravenously twice daily for 3-4 days) administered at the start of the febrile reaction has been recommended.¹⁵ In wildlife species, a long-acting intramuscular oxytetracycline antibiotic might prevent clinical disease if administered at the time of suspected exposure (i.e., *Amblyomma* spp. ticks noted on an animal) and before clinical signs.

The control of heartwater varies significantly between those regions where heartwater is endemic and those regions free of *E. ruminantium* and *Amblyomma* spp. ticks. Maintenance of endemic stability through vaccination and strategic tick control is instrumental for controlling clinical cases in areas where heartwater occurs. In those regions free of heartwater, tick control and regulation of animal movements is paramount for maintaining ticks and animals free of *E. ruminantium* infection.

Vaccination has historically relied on the intravenous administration of virulent infected blood, with subsequent treatment at the start of the febrile period. These "vaccinated" and treated animals are subsequently carriers. This method of vaccination is not practical for wildlife hosts in most situations. Although the development of inactivated vaccines has been complicated by field isolates differing in immunogenicity, a recent inactivated *E. ruminantium* vaccine was shown to decrease mortality, but not carrier state, in sheep challenged with heterologous strains from laboratory and field tick challenges.¹⁸

PREVENTION

Tick control is the backbone of heartwater prevention in heartwater-free regions, because animals originating in endemic areas may bring *Amblyomma* spp. ticks and the rickettsial organism when moved into the region. Precautions against transport of ticks must

be taken in countries (regions) of origin and import. Animals should be treated with appropriate acaricides both before export and again while in quarantine.

In recent years it has become evident that the reptile trade has been responsible for a number of exotic tick species, including *Amblyomma* spp., being imported into the United States.⁷ Reptiles imported into heartwater-free countries, such as the United States, from heartwater-endemic regions pose a significant threat of introduction. For example, a leopard tortoise (*Geochelone pardalis*) imported from Zambia to Florida had *A. sparsum* ticks that were PCR positive for *E. ruminantium*, demonstrating the introduction of the tick and rickettsial agent onto the American mainland.⁸ Because of this threat, in 2000 the United States instituted a ban on the importation of three of the most likely African tortoise species to introduce exotic *Amblyomma* spp. ticks: leopard tortoise, African spurred tortoise (*Geochelone sulcata*), and Bell's hinge-backed tortoise (*Kinixys belliana*).³ The use of a permethrin acaricide has been shown safe and effective for application to tortoise species and should be considered for the treatment of any tortoises at risk of being infested with *Amblyomma* spp. ticks.⁶

In addition to the introduction of *Amblyomma* spp. ticks to new geographic regions, the introduction of *E. ruminantium* is possible by the movement of subclinically infected domestic livestock and wildlife species. The difficulty in diagnosing infection in these carrier animals increases the risk associated with their movement between heartwater-endemic and heartwater-free regions. The only diagnostic test shown to be able to detect subclinical *E. ruminantium* infections is the PCR assay.¹⁷ Therefore, it is recommended that all species coming from a heartwater-endemic region and known to be susceptible to infection with *E. ruminantium* be tested for *E. ruminantium* by the PCR assay. Those animals testing positive should be refused entry into any heartwater-free area.

A similar threat of introduction of *E. ruminantium* and *Amblyomma* spp. ticks to heartwater-free regions is from the movement of free-ranging wildlife. For example, there is strong circumstantial evidence that much of the recent Caribbean interisland spread of *A. variegatum* has occurred through the movement of infested migratory birds, in particular cattle egret (*Bubulcus ibis*). The potential for cattle egret to introduce *A. variegatum* ticks and *E. ruminantium* into the United States was demonstrated in the early 1990s when a cattle egret, banded on the heartwater-endemic island of Guadeloupe, was found in Long Key, Florida.⁹

In addition to being one of the major limiting diseases for livestock production in much of sub-Saharan

Africa, heartwater is a significant threat to livestock and wildlife species both in Africa (e.g., wildlife translocated between heartwater-free areas and endemic areas for stocking of game parks) and in regions presently free of the disease (e.g., introduction of tick vectors or carrier animals). The indiscriminate host range of the rickettsial organism and its vectors, as well as other epidemiologic factors, makes heartwater a disease of worldwide importance. For example, the risk of introducing heartwater onto the American mainland is heightened by the presence of competent *Amblyomma* spp. tick vectors and the widely distributed, large population of white-tailed deer, a species demonstrated to be susceptible to heartwater.¹¹ Zoo and wildlife veterinarians, working with captive and free-ranging wildlife, must be alert to this disease and strive to minimize the threat it poses to livestock and wildlife populations.

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The Nutrition of “Browsers”

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Historically, “browsers”—whether leaf-eating primates, browsing ruminants, or browsing rhinoceroses—have often been considered difficult to maintain under conventional zoo feeding regimens. Although epidemiologic studies are generally lacking, several problems seem to have a high prevalence in browsing species. Browsing ruminants seem to be particularly susceptible to rumen acidosis in captivity and also to “ill thrift” and poor body condition in general.¹¹ In two large representative samples, the latter observation has led to explicitly recognized “syndromes”: the “wasting syndrome complex” in moose (*Alces alces*)^{9,69} and the “peracute mortality syndrome” or “serous fat atrophy syndrome” in giraffe (*Giraffa camelopardalis*).^{31,61} In tapirs (*Tapirus* spp.)⁴⁸ and langurs,⁵⁶ a soft fecal consistency is frequently observed, and browsing arboreal foregut fermenters, such as sloths or langurs, have a high prevalence of gastrointestinal (GI) upsets.^{25,26}

The objective of this chapter is to outline common denominators of “browsers” that need to be considered when developing management plans for browsing species.

BROWSERS ARE HERBIVORES FIRST

Browsers are herbivorous animals that feed, in the wild, predominantly or exclusively on dicotyledonous plant material, including the leaves and twigs of trees and shrubs, herbs, and forbs, but also wild fruits. This definition applies to a variety of reptile, bird, and mammal species. Among the mammals, species from different taxonomic groups, such as marsupials, rodents and lagomorphs, primates, edentates, artiodactyla and perissodactyla, are typically referred to as “browsers.” Within these groups, “browsers” are usually contrasted to other feeding types.

However, all browsers are not similar in feed choices, and these internal categories may have implications for appropriate feed types. In taxa where no “grazers” (species feeding predominantly or exclu-

sively on monocot plant material) exist, such as the primates or the edentates, “browsers” are often termed *folivores* (“leaf eaters”) as opposed to omnivorous species. The term “folivore” is not used in taxons in which both “browsers” and “grazers” exist, because some grazers also ingest preferentially the blades (“leaves”) of grass.

Vertebrates cannot digest plant cell walls auto-enzymatically. Therefore, herbivorous animals have to rely on the fermentation activity of symbiotic gut bacteria for the digestion of plant cell walls.⁶⁸ These bacteria are contained in one or two major fermentation sites within the GI tract: in a foregut, a hindgut, or both.

The basic challenge in herbivore nutrition is to maintain a healthy, stable gut microflora in the herbivorous animal. The most common problem in herbivore nutritional management is a relative lack of plant cell wall material (fiber) and a relative oversupply of easily digestible and fermentable substrates (mostly soluble carbohydrates, e.g., sugar and starch; in extreme cases, perhaps protein). Such an oversupply will lead to direct disturbances of the microflora in foregut fermenters (comparable to rumen acidosis in domestic ruminants). In hindgut fermenters, these substrates are primarily absorbed from the small intestine before reaching the hindgut fermentation site, where they will only cause disturbances (comparable to cecal acidosis in domestic horses, the major cause of laminitis) if given in particular oversupply. This dualism may be illustrated with two primate groups: in captivity, with an oversupply of easily fermentable carbohydrates in conventional diets, langurs (foregut fermenters) have a history of digestive upsets and malnutrition,²⁶ whereas lemurs (omnivores and some hindgut-fermenting herbivores) have a history of being obese.⁶⁸ Similarly, among the ungulates, foregut-fermenting browsers such as giraffe or moose have a history of poor body condition in captivity,^{9,62} whereas hindgut fermenters, such as tapirs, rhinos, or elephants, are often overweight.^{1,60,75}

In zoos, browsers have been traditionally recognized as animals with a difficult nutritional management.

Partly, this reflects a basic problem in herbivore nutrition. As evolved omnivores, humans value easily digestible carbohydrates such as sugars and starches. We have a long history of cultivating grains and fruits for their respective starch and sugar content, and we intuitively want to include these items in the diets of animals we keep. Herbivores have also evolved to select for these items. In the natural environment, their sparse availability limits any potential danger of oversupply; in captivity, however, situations might exist in which the offer of these items is not limited. A restricted supply of such items, with a generous supply of high-fiber feeds, is therefore the fundamental approach to herbivore nutrition.

BROWSERS ARE DIFFERENT

Nevertheless, compared with other herbivores, browsers still appear to be particularly sensitive. Possibly, one

should not prioritize the investigation of other nutritional factors besides a high-fiber diet at first. In free-ranging browsers, a high fiber content is the major determining characteristic of their natural diet (Table 55-1); free-ranging browser diets are not distinctively "lower in fiber" than those of grazing species. In terms of fiber and carbohydrate content, a pelleted feed for "browsers" should therefore be suitable for "grazers" as well.

For the digestive physiology of many species, the provision of nonpelleted high-fiber diet items, that is, *forage*, is crucial. In ruminants, for example, the provision of structured fiber is a prerogative for proper rumen function, and in most herbivores, forage material is the only guarantee for a "normal" fecal consistency. In addition, forage addresses the most basic ethologic requirements of animals that have evolved physiologic and psychologic adaptations for the handling of (complex) food items, and oral stereotypies have been observed in many species such as cattle,⁶⁴

Table 55-1

Fiber Content of Natural Diet of Different Free-Ranging Herbivores*

Species	Crude Fiber (% Dry Matter)	NDF† (% Dry Matter)	Source (Reference)
Giraffe (<i>Giraffa camelopardalis</i>)	—	50-70	(61)
Okapi (<i>Okapia johnstoni</i>)	—	43-48	(59)
Moose (<i>Alces alces</i>)	20-45	50-70	(5)
White-tailed deer (<i>Odocoileus virginianus</i>)	—	35-50	(79)
Duikers (various spp.)			(24)
Forage		5-70	
Fruits		30-60	
Buffalo (<i>Syncerus caffer</i>)	30-40		(30)
Waterbuck (<i>Kobus ellipsiprymnus</i>)	30-40		(80)
Black rhino (<i>Diceros bicornis</i>)	20-60	40-70	(4)
White rhino (<i>Ceratotherium simum</i>)	36	75	(47)
African elephant (<i>Loxodonta africana</i>)	35-50	60-70	(53)
Three-toed sloth (<i>Bradypus tridactyla</i>)		40	(31)
Colobus monkeys (different species)			(49, 55)
Forages		30-70	
Fruits		50-70	
Ring-tailed lemur (<i>Lemur catta</i>)		30-50	(22)
Howler monkey (<i>Alouatta aloutta</i>)			(70)
Forages		20-80	
Fruits		20-70	
Gorilla (<i>Gorilla gorilla</i>)		40-80	(65)
Orangutan (<i>Pongo pygmaeus</i>)		40-60	(34)
Browsing kangaroo (<i>Macropus fuliginosus</i>)		60-80	(21)
Grazing kangaroo (<i>Macropus rufus</i>)		50-80	(21)

*Note that free-ranging browser diets (even fruits) do not have particularly lower fiber levels than those of other herbivores.

†Neutral detergent fiber, a measure of cell wall (fiber) content.

okapi and giraffe,⁴² and horses⁸¹ eating forage-deprived or low-fiber diets. Particularly with respect to the suitability of forage, browsers are different: in contrast to the situation with grazing herbivores, the staple provision with a forage that is readily accepted by browsers without causing GI problems may be challenging in certain species.¹¹

This general discrepancy between grazers and browsers is reflected in the traditional recipes for pelleted feeds provided by commercial manufacturers. Although there is no indication that browsers are adapted to a higher fiber intake than grazers, experience has led to the development of particular “browser” pellets high in fiber. In contrast, “regular herbivore” or “grazer” products contain less fiber because these animals readily accept the staple fiber source offered to them in addition to the pellets: grass hay. Using the product ranges of both U.S. Mazuri (PMI Nutrition International, St. Louis, Mo) and U.K. Mazuri (SDS, Essex),* it may be demonstrated that the recipes for ungulates increase in fiber content according to the difficulty of providing the target species with readily acceptable forage material, with moose being recognized as a large browsing species particularly reluctant to accept hay (Table 55-2).

It should be noted that the fiber levels reported in Table 55-2 are still in the lower range of the fiber levels reported in diets of free-ranging animals (see Table 55-1). Interestingly, this discrepancy is even greater if the primate diets of commercial manufacturers (Table 55-3) are compared to the diets of free-ranging primates (see Table 55-1).

This chapter contrasts selected peculiarities of browsing and grazing herbivores, which are adaptations to certain characteristics of their natural diets, and links this with health problems in captivity and problems in diet design for captive animals. In the absence of systematic research on the nutritional managements of browsers, including normal gut microbiology, the suggestions for feeding browsers must, by necessity, remain speculative.

DIFFERENCES BETWEEN GRAZERS AND BROWSERS

A fascinating multitude of anatomic and physiologic differences has been postulated (and sometimes demonstrated) between browsing and grazing herbi-

vores and their respective diets.¹⁸ Internal and external differences in muzzle width, tooth form, salivary gland size and saliva composition, and GI tract morphology have been suggested as underlying features of importance in differential nutrition of browsing herbivores.

Table 55-2

Declared Crude Fiber Content of Select Herbivore Feeds from Catalogs of Two Commercial Suppliers*

Diet Name	Crude Fiber (% Dry Matter)	NDF† (% Dry Matter)
Herbivore 16-ADF ¹	16.7	32.2
Herbivore 25-ADF ¹	25.6	43.4
Browser breeder ¹	27.8	43.6
Browser maintenance ¹	31.1	48.1
Moose maintenance ¹	35.6	54.8
Grazer ²	11.2	
Browser breeder ²	18.6	
Browser maintenance ²	21.4	
Moose ²	24.0	

*Note that fiber levels do not reflect differences in “fiber requirements” between target species, but that fiber content increases with the recognized target species’ reluctance to accept grass or alfalfa (lucerne) hay forage.

†Neutral detergent fiber, a measure of cell wall (fiber) content.

¹Mazuri (PMI, St Louis, Mo USA).

²Mazuri (SDS, Essex, UK).

Table 55-3

Declared Crude Fiber Content of Select Primate Feeds

Diet Name	Crude Fiber (% Dry Matter)	NDF* (% Dry Matter)
Leaf-Eater Primate diet ¹	15.6	27.4
Primate High Fiber Sticks ¹	16.1	32.9
Primate Browse Biscuit ¹	17.8	29.4
Leaf Eater Primate ²	13.8	
High-fiber primate diet ³	10.0	
Leaf Eater Red Apple ⁴	14.4	20.8

*Neutral detergent fiber.

¹Mazuri (PMI, St Louis, Mo USA).

²Mazuri (SDS, Essex, UK).

³HMS (Bluffton, Ind USA).

⁴Marion Zoological (Plymouth, Minn USA).

*The use of product information from commercial suppliers has solely didactic purposes and does not imply a particular recommendation or warning.

Not all these differences are of direct relevance for zoo animal feeding. The following differences, however, most likely are important for the nutritional management of browsers:

Chemical Composition of Forages

Browse is regularly reported to contain more protein than grasses.¹⁸ On the one hand, this is most likely due to analytic difficulties: protein content is usually assessed by analyzing nitrogen and multiplying by the factor of 6.25. However, browse may contain significant amounts of nonproteinaceous nitrogen in secondary plant compounds, and it has been suggested, at least for tropical browse, that the true conversion factor for the calculation should be as low as 4.4.⁵⁴ Lignin also contains nitrogen in a chemically unavailable form.⁷⁷ Therefore, bound nitrogen may erroneously contribute to higher protein values reported for many browses unless available versus bound protein fractions are analyzed separately. On the other hand, high reported protein contents should not automatically lead to the assumption that browsers have higher protein requirements.

A classic case of an assumed high protein requirement in a browser is that of giraffe. It was suspected that low-protein diets play an important role in the serous fat atrophy syndrome (peracute mortality syndrome) observed in captive giraffes,³² and high protein levels of 18% dry matter (DM) were consistently recommended for this species. However, it was later reported that the problem also occurs in animals with "adequate" protein provision.⁴⁵ A comparative evaluation of experimentally determined protein requirements in ruminants does not reveal relevant differences between the different feeding types.¹¹ The fact that browsers do have higher fecal and urinary nitrogen losses when kept on browse does not reflect higher true endogenous losses, but rather is caused by the secondary plant compounds in the browse fed. If the same animals are kept on a diet without secondary compounds, the nitrogen balance is "back to normal."⁶⁶ As a logical consequence of such considerations, the recommended protein levels for giraffe, for example, have recently been reduced to 12% DM.³⁴ Particularly high protein levels for browsers appear unnecessary.

Browsing ruminants have traditionally been termed "concentrate selectors."⁴⁰ This may have led to a widespread conception that browsers particularly prefer (and require) easily digestible carbohydrates such as starch and sugars, and that such animals may receive

higher proportions of concentrate feeds. However, comparative evaluations of ruminant necropsies have revealed a higher prevalence of GI disorders, particularly acidosis, in browsers compared with grazers. Evidently, the so-called concentrate selectors often suffer from a condition triggered by too much "concentrate feeds."¹¹ Therefore, for didactic reasons alone, the term "concentrate selector" should be avoided. Actually, there is no indication that browse has a higher sugar or starch content than grass; however, browse contains higher proportions of soluble fibers, such as pectins.⁶⁶ Pectin sources have been recognized in domestic ruminant nutrition as high-energy "concentrates" that may favorably replace starch-containing grain products because of a significantly less acidotic potential compared to grains.⁷⁸ Therefore, pectin sources may be considered excellent energy-supplying diet items, both for browsers and grazers alike.^{41,46}

The fiber component of browse is generally more lignified than that of grasses.⁷⁷ *Lignin* is a basically indigestible material, and thus more highly lignified fiber is less digestible. Broad surveys of various forages have shown lignification indices (% lignin/% neutral detergent fiber [NDF]) approaching 20% to 30% of total fiber in browses, compared with perhaps half that proportion in grasses. To date, no observations indicate that a deliberate inclusion of lignin, rather than a general increase in overall fiber levels, is of particular health relevance for browsing animals. However, it is most certainly associated with decreased forage digestibility in a variety of herbivore species.

Browse plants, in particular the leaves of woody plants, often contain secondary plant compounds that may act as digestibility reducers or toxins that may serve as feeding deterrents. As adaptations, browsing animals may produce salivary proteins that reduce the effect of such substances (e.g., the tannin-binding proteins), and are likely to have evolved a variety of metabolic detoxification mechanisms.⁶ Because of the enormous variety of secondary plant compounds, general rules are difficult to distill from the literature. Some positive effects of some of these compounds have been reported (e.g., as antioxidants or anthelmintic substances, or by "protecting" dietary protein from ruminal degradation and thus enhancing intestinal digestion). In roe deer (*Capreolus capreolus*) fawns, an increased food conversion and a tendency for higher circulating antioxidant levels have been reported on a pelleted diet with added tannin.¹² In black rhinoceros (*Diceros bicornis*) feces, the antioxidant capacity was higher on tannin-containing diets.¹⁷ However, the experimental evidence is still extremely limited. More studies are needed before any recommendations about

the deliberate inclusion of such substances may be made.

Fermentation Characteristics

In the few published *in vitro* studies, browse material *reached its maximum of fermentation sooner* than grass material, most likely because of a higher content of slowly fermenting cellulose fiber in grass and more rapidly fermenting pectin and indigestible lignin fiber in browse.⁴³ This has important implications for GI physiology. In theory, it would not make sense for a browser to retain ingesta as long as a grazer does; compared with grass, browse does not yield relevant amounts of energy after a certain digestion time. Indeed, there have been numerous indications that browsing ruminants and browsing rhinos have shorter ingesta retention times than their grazing counterparts,^{16,19} but for a comprehensive comparative evaluation, the existing database is still too small.

For the design of zoo animal rations, this could mean that browsers should not be able to digest any given forage as efficiently as a grazer (due to shorter retention times, *i.e.*, less time available for digestion). Therefore, if a browser accepted a grass hay diet, for example, it might have to ingest more of it to achieve a similar uptake of digestible energy as a grazer of comparable size. In ruminants, shorter retention times within the rumen will also translate into less degradation of substances susceptible to bacterial fermentation; for example, polyunsaturated fatty acids are hydrogenated to a lesser degree, and one would expect less bacterial transformation of proteins and less degradation of vitamin A or vitamin E.⁷

However, these characteristics are unlikely to be of practical relevance; one could save on vitamins, or on “protected proteins,” in browser diets. As long as quantitative information on these effects is lacking, however, it is advisable to keep vitamin levels as high as in domestic (grazing) ruminants. The impact of rumen-protected proteins has not been examined in zoo ungulates and probably has more applications, particularly economic, for livestock species.

Physical Characteristics

The physical differences between grass and browse are suspected to be responsible for the widespread reluctance among browser species to ingest grass hay, and in some cases perhaps even alfalfa hay, in similar amounts as grazers.¹¹

Many grasses defend themselves against herbivores by abrasive silica. Browse is generally less abrasive and contains less acid-insoluble ash. As adaptations, grazers of all taxons have a hypsodont dentition (high-crowned teeth) that may be worn down throughout their lifetime, whereas browsers mostly have low-crowned teeth.¹⁸ For free-ranging browsers such as moose, it has been postulated that the animals should select against an abrasive diet,³⁶ and for roe deer, it was shown that the diet selected contained less silica than the available forage.⁷⁶ For captive browsers, even if only partly fed on grass hay, this should mean that after years in captivity, they should display more severe tooth wear than grazers. In this respect, unnatural tooth wear has been identified as an important problem in a captive browser, the giraffe.^{20,28}

Differences in fracture properties between forages—with browse being more brittle (requiring crushing dentition) and fractionating into more polygonal particles, and grass being more flexible (requiring cutting dentition) and fractionating into longer, fiberlike particles—have thus far only been sparsely documented.¹⁸ Nevertheless, these differences have most likely driven the evolution of tooth shape and also gut anatomy. It may be expected that the respective dentitions of browsers and grazers are adapted to finely comminute their respective natural diets. In comparative assays, this should mean that browsers do not chew grass hay/conventional zoo diets into as fine particles as grazers do (documented in ruminants¹⁰ and macropods⁵⁰), and that the feces of free-ranging browsers should contain finer particles than those of captive specimens. The latter hypothesis has been confirmed for tapirs,⁴⁸ even though the captive animals ingested a high proportion of finely ground “concentrates.” Differences in fracture properties, with an increased tendency of grass material to form a “fiber mat” in the rumen (a prerequisite of efficient particle retention in domestic ruminants), have been linked to the reluctance of browsers, whose rumen lacks the strong musculature of grazers, to ingest grass material in large proportions.¹³

Certain Forages Are Inappropriate for Browsers

In conclusion, browsers should have evolved to avoid grass because of the abrasiveness of this diet, both grass and (to a certain extent) alfalfa because of problems in food comminution from an unsuitable dentition, and problems in ruminal food processing because of unsuitable rumen morphology. Reluctance to ingest grass or even alfalfa hay has been documented in

numerous ruminant browsers,¹¹ in macropod browsers,⁵⁰ in the browsing suid babirusa (*Babyrussa babyrussa*)⁵¹ and is also reflected in the well-known reluctance of many tapirs to ingest such forages. In contrast, Marcus Clauss has seen black rhinoceroses readily ingest grass hay and thrive on grass hay-based diets for long periods, but with excessive tooth wear. Intuitively, grass and alfalfa are rarely offered to those browsers termed "folivores" (sloths or primates), except the largest apes (gorillas).

If browsers are fed on such inappropriate forages, the following two consequences are to be expected:

1. Low acceptance of the forage, resulting in an unintended, high proportion of "concentrate" feeds, which may result in acidosis (particularly in foregut fermenters). This has been reported in ruminants¹¹ or suspected in colobines,⁷³ with associated laminitis, liver abscesses, oral stereotypies, or poor body condition; or resulting in obesity (particularly in hindgut fermenters); and generally in poor fecal consistency.
2. If the forage is accepted, increased tooth wear (in the case of grass) and a less effective particle reduction, resulting in an increased risk of phytobezoar formation, as reported in giraffe or mule deer.¹¹ In this context, even certain browse species may be inappropriate for animals adapted to different browse items (e.g., acacia-induced phytobezoars in langurs, but note that phytobezoars must also frequently occur in free-ranging langurs⁵⁷).^{3,29}

To our knowledge, the long-term consequences of these effects have not been assessed; however, in individual browsing species, short life spans in captivity have been noted, including the well-documented mortality peak in captive moose at 6 to 8 years of age⁹ and the high mortality in captive langurs²⁶ and sloths.^{25,63}

PRACTICAL FEEDING

Pellet Design

Historically, the development of the aspen sawdust-based, pelleted ration for moose at the Kenai Moose Research Center in Alaska⁶⁷ is the hallmark of a major breakthrough in the nutrition of captive browsers (NDF = 57% DM). The success of this ration was probably not a result of the woody component itself, but simply because the sawdust ingredient ensured a high fiber content of the final product.¹⁴ Ironically, the primary incentive for the use of the sawdust ingredient

seemingly was not an increase of the overall fiber level; rather, it was argued that lignified forage has different fracture properties than grass. However, sawdust is a poor source of lignin (but rather a good source of cellulose), and in a milled and pelleted compound feed, the original fracture properties of the ingredient materials are of no relevance.

In subsequent steps the recipe of the pellet was refined⁶⁹; the most important refinement probably was the inclusion of beet pulp as a pectin-rich energy source and a corresponding decrease in or exclusion of grain (including corn) products. The use of beet pulp as a pectin source instead of grains in the nutrition of herbivores has been advocated increasingly over the past decades⁷⁸ and has been tested successfully with moose, giraffe, and okapi.^{44,46,69}

Basically, a safe pellet for any herbivore, including browsers, should be based on a forage meal, whether sawdust, alfalfa meal, grass meal, sunflower or soy hulls, cellulose powder, or mixture of these, to ensure a high fiber content; should use unmolassed beet pulp as an energy source; and should not include significant amounts of grains or corn. Linseed products might be useful to increase the omega-3 fatty acid content,^{8,72} especially if soy products are included to increase protein levels, and sodium bicarbonate may also be used as a buffer further to prevent acidosis.² A practice to use (and market) such a pellet for browsers only, but not for grazers, cannot be based on physiologic considerations. However, because animals will need higher amounts of such high-fiber, low-energy feeds, grazers might, for financial reasons, receive restricted amounts of more energy-dense feeds of lower fiber content, under the assumption that they will readily consume the staple hay item.

Table 55-4 provides two examples of recipes for pelleted diets.

Forage Choice

The major challenge in the nutrition of browsers is to find a forage material that is both suitable and readily accepted by the animals and logistically easy to acquire. For most browsing species, alfalfa hay is used as a surrogate, although chemically it may not be the best nutrient substitute. The chemical composition of alfalfa hay differs among the continents, with mostly early-bloom alfalfa used in the United States, and differs from browse material as well (Table 55-5).

In particular, an exclusive consumption of alfalfa leaves only could, in combination with a low-fiber pelleted compound, result in a diet of comparatively high

Table 55-4**Example Recipes for Pelleted Diets Fed to Browsers***

Ingredient	% Original Weight
Example 1	
Sawdust	22.5
Beet pulp	20.0
Canola meal	20.0
Alfalfa meal	10.0
Sucrose	5.5
Soybean meal	5.0
Dried orange peel	5.0
Mineral/vitamin supplements	12.0
NDF† (% dry matter)	42.7
Example 2	
Beet pulp	22.5
Soy extraction meal	22.5
Alfalfa meal	22.5
Sunflower hulls	12.5
Wheat	8.0
Molasses	2.5
Cellulose powder	2.5
Linseed	2.0
Sodium bicarbonate	1.0
Mineral/vitamin supplements	4.0
NDF† (% dry matter)	40.7

From Berndt C, Klarenbeek A, Clauss M, et al: *Proc Eur Assoc Zoo Wildl Vet* 5:371-372, 2004; and Schochat E, Robbins CT, Parish SM, et al: *Zoo Biol* 16:479-494, 1997.

*Suitable for grazers as well.

†The calculated/analyzed neutral detergent fiber (NDF) value is given as well for comparison with Tables 55-1 and 55-2.

protein and low fiber levels. Excessively high protein contents could, for example, lead to the formation of copper-sulfide bonds in the rumen, thus reducing copper availability.^{23,74} For example, a low copper status has been observed in duikers, small ruminants that selectively ingest the leaves, but not the stems, of the alfalfa hay offered.³³

In Europe, alfalfa is much less common in dairy or beef rations and thus much more difficult to acquire for many zoos, as reflected in the ranges for alfalfa (lucerne) hay from Europe with high fiber and low protein levels (see Table 55-5). Direct contracts with local farmers might be a good alternative in such situations. However, the provision of browse material itself in large quantities should be the ultimate goal of

any zoo interested in browser husbandry, even though this goal implies logistical and nutritional challenges.

From a logistical point of view, browse is labor intensive to harvest, difficult to store, only seasonally available in the temperate zone, and often not available in large amounts. With regard to availability, zoos with browser breeding programs should either initiate cooperation with the local forestry agencies, with the result of a constant supply, or establish a browse plantation of their own.³⁹ Browse may be harvested on a daily or weekly basis and may be dried (even commercially available), frozen, or ensiled.³⁶ The acceptance of the different conservation methods may vary among species.

From a nutritional point of view, the use of browse infers a high degree of uncertainty, especially in regard to potentially toxic secondary plant compounds, which may vary seasonally or geographically, even within the same plant species. Therefore, the reasonable admonition is that the feeding of browse should be based on scientific recommendations.⁵⁸ In practice, such a demand is difficult to fulfill because of the variety and variation in secondary compound contents, even within the same plant species. Additionally, experiences with cases of plant poisoning and the uneventful feeding of browse may be used; recently, such observations have become more easily accessible by the establishment of browse databases (e.g., www.foragerssource.org). In any case, it is advisable to offer a variety of (reportedly harmless) browse species because this will increase overall intake and allow the animal to select among different items (all of high fiber content), and the ingestion of smaller amounts of different secondary compounds is usually considered less dangerous than the ingestion of a larger amount of any one particular toxin.

Ration Composition

Following widespread (but not always heeded) recommendations, rations of browsers, as with other herbivores, should not contain rapidly fermentable carbohydrates, such as the sugars and starch contained in commercial fruits and grain products, including bread and grain-based pellets.⁵⁸ Instead, rations should be composed of a high-fiber pellet and adequate roughage, such as grass and alfalfa hays (in ungulates) or green leafy vegetables with stem fractions (in primates).

The major problem in ration design for browsers and other herbivores is that the amount of forage offered, whether browse, alfalfa hay, or grass hay, is

Table 55-5

Composition of Selected Nutrients in Alfalfa Hay (*Medicago sativa*)*

Feedstuff	% NDF	% Crude Fiber	% Crude Protein	% Ca	% Mg
Alfalfa	43	25	22	1.6	0.4
Early bloom (USA) ¹					
Alfalfa	47	28	19	1.4	0.4
Midbloom (USA) ¹					
Alfalfa	52	32	14	1.3	0.3
Late bloom (USA) ¹					
Alfalfa leaves only ¹	34	18	23	2.6	0.4
Lucerne hay (UK) ²	46		18	1.5	0.3
Lucerne hay (Europe) ⁴	36-69	23-43	12-19	0.9-3.8	0.1-0.2
Temperate browses					
Browse leaves ³	27-66		13-23	0.3-3.0	0.1-0.3
Browse leaves ⁴	43-54	13-26	14-18	1.4-2.1	
Browse twigs ³	63-82		3-7	0.5-2.6	0.1-0.2
Browse twigs ⁴	67-75	19-42	3-7	1.0-1.5	

*Harvested in the United States (USA) and United Kingdom (UK), and comparison with alfalfa leaves that may be selectively consumed by browsing herbivores compared to some temperate and native browses. All values on a dry matter (DM) basis. NDF, Neutral detergent fiber; Ca, calcium; Mg, magnesium.

¹US-Canadian Tables of Feed Composition, NRC.

²UK Zoo Feed Ingredient Database; Fidgett A: Unpublished Data.

³Zootrition 2.5 database, St Louis, Mo USA.

⁴Data from references 4 and 5.

much more difficult to estimate than the amount of pellets or produce given. As target amounts of different food items, those given in the Nutrition Advisory Group (NAG) fact sheets for ungulates⁵² and leaf-eating primates²⁷ may be used, and the amount of forage indicated in these guidelines may be replaced in part by browse. However, to control whether these target intake values are actually reached in a specific animal or animal group, sporadic weighing of foods offered and left over, possibly with a DM determination of a representative subsample, is necessary. Furthermore, restricted feeding of some preferred, but less nutritionally balanced, items may be an important aspect of nutritional management to encourage fiber consumption.

Browse material is difficult to quantify because it is generally offered as branches, the majority of which is not edible for most species. A weighing of a total branch offered therefore provides little information about the amount of edible browse offered. This problem is evidently easy to solve in the case of silages, or plucked leaves. In the case of whole branches, a rough estimation of the edible leaf and twig mass may be made using the diameter of the branch at the point of cutting and species-specific allometric equations.¹⁵

An important question for many zoos involves the quantity of browse to include in browser diets. To

date, no scientific answer exists, and anecdotal experiences are insufficient to distill a rule of thumb. In captive giraffes it has been documented that the overall DM intake increased in one particular group with the addition of up to 3 kg of fresh, edible browse (i.e., not including large branches) compared with a diet without or with only 1 kg of browse. In contrast, the addition of 6 kg of the same browse material did not increase the overall intake any further.³⁷ However, the browse was always consumed completely, and total DM intake is only one parameter of interest; in particular, long-term effects of such a feeding regimen are difficult to document.

The use of safe browse species should be decided on the basis of data on the intake of the different diet items offered to the animals. In browsers, if the intake of the offered conventional forages is lower than recommended, the proportion of browse in the overall diet should be increased.

CONCLUSION

Moderately digestible, high-fiber and moderate-protein diets best duplicate—from a chemical point of view—the foodstuffs consumed by most browsers in nature,

whether highly frugivorous, folivorous, or woody plant-part consumers. Many of the pellets currently fed to captive species are low in fiber and high in starch and protein, which may lead to health problems such as acidosis and mineral/electrolyte imbalances. Native chemistry should provide clues for more suitable captive diet formulations. Low-fiber foods containing sugars or starch, such as most domesticated fruits and grains, should not be used (except perhaps for training purposes).

Enrichment should be provided by variety of high-fiber forages, not by offering sugary or starchy items. The inclusion of tanniniferous foods in artificial diets should not be regarded as exclusively negative, and effects of tannins on animal response should be evaluated further. Incorporation of palatable, readily accepted sources of dietary fiber, to maintain digestive tract function and psychologic well-being, should be considered the highest priority in feeding all browsers. Facilities aiming for long-term browser husbandry should incorporate contingency plans for the provision of such forage materials into their overall management approach.

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Side Effects of Etorphine and Carfentanil in Nondomestic Hoofstock

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Immobilization of nondomestic hoofstock species presents unique challenges and risks to the zoo and wildlife veterinarian. The successful outcome depends on careful planning of the anesthetic event and consideration of factors that may adversely affect the success of anesthesia, especially the conditions surrounding immobilization (e.g., captive vs. free-ranging animals, environmental temperature, size and type of enclosure), as well as knowledge of the species to be immobilized and their often unique response to specific immobilizing agents.

To minimize morbidity and mortality and safely and effectively immobilize ungulates, induction of anesthesia should be rapid and without excitement. During immobilization, adequate muscle relaxation, good analgesia, and minimal cardiopulmonary depressant effects should be present. Recovery should be rapid, smooth, and complete after administration of the reversal agent(s). There is no ideal protocol, and each immobilization must account for many variables, including the number and experience of the personnel, the available equipment to monitor the immobilized animal effectively, and most importantly the experience and skill of the veterinarian performing the immobilization. Some species, such as giraffes, present unique challenges because of their size and anatomic features (e.g., long neck).²

A variety of different anesthetic agents have been used with variable success for the immobilization of captive and free-ranging nondomestic hoofstock species. In most cases, potent opioid agents such as carfentanil citrate, etorphine hydrochloride (M99), and more recently thiafentanil (A-3080) are used either alone or more often in combination with synergistic agents such as α_2 -adrenergic agonists (e.g., xylazine, medetomidine) and dissociative agents (e.g., ketamine hydrochloride) to facilitate rapid induction of anesthesia. The addition of synergistic agents allows for a lower dose

of the opioid agent and a reduction of side effects, such as possible excitement during induction of anesthesia, poor muscle relaxation, and hypertension during immobilization. In most cases, administration of specific antagonists facilitates rapid, smooth, and complete recovery.

Before selecting an anesthetic protocol, it is mandatory to be familiar with the anesthetic side effects of the agent(s) being selected and be aware of species-specific reactions to the drugs being used. There are pronounced species differences in response to opioid agents and their antagonists. A combination that works effectively in one species may not work in another and may result in poor quality induction, severe cardiopulmonary depression, incomplete or stormy recovery, including renarcotization.

This chapter details side effects, corrective measures, and monitoring techniques to immobilize nondomestic hoofstock effectively with opioid agents.

OPIOID AGENTS

In most ungulate species, administration of potent opioid agents (etorphine, carfentanil, thiafentanil) in combination with synergistic agents (e.g., xylazine, medetomidine, ketamine) will produce rapid immobilization, adequate muscle relaxation, analgesia, and in most cases, rapid recovery after administration of specific antagonist. Opioid agents have a wide margin of safety and are fast acting, but they also cause central nervous system (CNS) depression, poor muscle relaxation if used alone, and pronounced cardiopulmonary depression.^{11,19} Species differences exist in the sensitivity to opioid agents, and administration of these agents alone is often associated with excitement, myopathies, regurgitation, and tachypnea/tachycardia or, depending on the species, bradycardia/bradypnea.

Arterial hypotension or more often hypertension is seen after opioid administration, and species differences are apparent. All opioids are excreted by the kidneys after hepatic metabolism.

Worldwide, *etorphine hydrochloride* (M99) is the most common opioid agent used for immobilization of hoofstock. Etorphine may be used either alone or in combination with neuroleptic synergistic agents. Induction times with etorphine are usually longer compared with other opioid agents, such as carfentanil. The most common side effect associated with etorphine immobilization is pronounced respiratory depression, but common opioid effects (e.g., excitement, poor muscle relaxation, bradycardia or tachycardia, renarcotization) may be seen after administration of the opioid antagonist.

Carfentanil is a potent synthetic opioid with a morphinelike mode of action. It is more potent than etorphine and has a longer duration of action than etorphine and thiafentanil. Carfentanil is approved as an immobilizing agent for cervidae and provides good analgesia during immobilization. As with all opioids, carfentanil is fast acting and has been used in numerous hoofstock species for rapid induction of anesthesia.^{4,5,10,20,21} When used alone, carfentanil is rapidly absorbed and often associated with hyperexcitability during induction, poor muscle relaxation, tachycardia, hypertension, tachypnea, regurgitation, and prolonged recovery periods if an inadequate low dose of the antagonist is administered.^{18,20,21}

Thiafentanil oxalate (A-3080) is a synthetic, short-acting fentanyl derivative with less potency than carfentanil and has been used successfully for the immobilization of various hoofstock species.^{7,8,14,24} Compared with carfentanil and etorphine, thiafentanil offers the advantages of shorter duration of action and fewer cardiopulmonary depressant effects. In most cases, cardiopulmonary parameters are maintained at physiologic levels.^{7,8} The effects of thiafentanil can be antagonized rapidly and completely with the opioid antagonist naltrexone hydrochloride, with no reports of renarcotization.⁹

PREANESTHETIC CONSIDERATIONS

Before immobilization, numerous factors (e.g., captive vs. free-ranging animal, isolated or within a herd) must be taken into account in selecting an anesthetic protocol that includes safe and effective dosages of the opioid and the synergistic agent(s). The health status of the animal, species-specific sensitivities to a partic-

ular drug combination, and available equipment for transport and monitoring should also be determined. The key for prevention of complications associated with immobilization is preparedness and careful planning of the procedure, including determination of the location where the animal will recover.

During recumbency, immobilized hoofstock, especially large species, are susceptible to the development of complications, which may include cardiopulmonary compromise, development of shunts, ventilation/perfusion mismatches, hypoventilation, and hypoxemia. In immobilized hoofstock, increased peripheral resistance and systemic hypertension are often seen.

ANESTHETIC AND CARDIOPULMONARY EFFECTS

The anesthetic effects of opioid agents, especially if used alone, are variable, dose dependent, and sometimes species specific. A key factor for the safe immobilization of captive and free-ranging hoofstock species is a rapid, smooth, and predictable induction of anesthesia. Again, good muscle relaxation and adequate analgesia should occur during immobilization, and cardiopulmonary parameters should be kept within physiologic ranges. Both carfentanil and etorphine fulfill most of these requirements, but if used alone, either may also cause hyperexcitement during induction, poor muscle relaxation, severe respiratory depression, especially at high dosages, and hypertension. Effects and quality of induction are dose dependent.²³ To decrease the opioid dosage, improve muscle relaxation, and maintain cardiopulmonary parameters, synergistic agents such as xylazine, medetomidine, and ketamine are often added to the anesthetic protocol.⁶ The quality of anesthesia, including muscle relaxation and recovery time, is species dependent and in some species better with etorphine combinations than carfentanil combinations.¹³

Physical trauma after drug administration may be encountered at any time during induction and recovery. The key to prevention of trauma is selection of an effective dose and drug combination that produces rapid immobilization and minimizes excitement. For rapid reversal of anesthesia, effective antagonists and dosages should be selected. Also, environmental conditions should be evaluated and any potentially hazardous objects within an enclosure removed.

All opioid agents cause respiratory depression, including a decrease in respiratory rate and tidal volume.^{17,20} Relative arterial oxygen saturation (SpO_2), as indicated by pulse oximetry, is typically less than 90%. Arterial blood gas (ABG) analysis often confirms marked to severe respiratory depression, as indicated by low arterial oxygen tension (PaO_2 ; <60 mm Hg) and arterial blood oxygen saturation (SaO_2) of less than 90%, confirming severe hypoxemia.²⁰ Arterial carbon dioxide tension (PaCO_2) values are often greater than 50 mm Hg, indicating pronounced hypercapnia. Decreased pH values indicate respiratory acidosis. The risk of mortality and development of capture myopathy is particularly increased in hyperthermic and hypoxemic ungulates because the oxygen demand by the tissue, especially muscle, cannot be met.

The effects of carfentanil, etorphine, and thiafentanil on cardiovascular parameters include decreases in heart rate, arterial hypertension or hypotension indicated by increase or decrease in systolic, diastolic, and mean arterial pressures, as well as potentially premature ventricular contractions, premature atrial contractions, and junctional escape rhythm. Dama gazelles (*Gazella dama*) immobilized with carfentanil alone were hypertensive after drug administration (systolic arterial pressure [SAP] >150 mm Hg), but increases were within ranges reported in other domestic and nondomestic ungulates.²¹ The ABG parameters in these gazelles were well maintained and did not indicate episodes of hypoxemia. Hypoxemia and hypercapnia were not observed, but these effects may be species and dose dependent.

Bongo antelopes (*Tragelaphus eurycerus isaaci*) immobilized with a carfentanil-xylazine combination showed decreases in heart rate throughout the immobilization period, whereas severe systemic hypertension (SAP >200 mm Hg) was noted after 45 minutes of immobilization and thereafter. In these antelopes, significant increases in plasma norepinephrine and decreases in plasma 3,4-dihydroxyphenylacetic acid concentrations have been associated with the development of hypertension.²⁰ Heart rates and systemic arterial pressures are often higher with carfentanil than with etorphine.¹³

Studies in domestic goats comparing the cardiopulmonary effects of carfentanil and etorphine demonstrated that both agents will cause increased systemic and left ventricular end-diastolic pressures as well as increased total peripheral resistance. A dose-dependent increase in left ventricular stroke volume, mean pulmonary artery pressure, PaO_2 , PaCO_2 , and body temperature was seen. Both etorphine and carfentanil induced hypertension, bradycardia, and bradypnea in

goats, and effects were seen more rapidly with carfentanil than with etorphine.¹²

In hoofstock species, administration of opioid agents will increase vagal tone and release of histamine, prolactin, and somatotropin. In addition, decreased motility of the gastrointestinal (GI) tract, increased bladder tone, and decreased tone of the uterus is typically present. Opioids raise the pain threshold, reduce the perception of pain, are effective in inducing analgesia, and are the drugs of choice for the treatment of acute and severe pain. However, the duration of action varies among species and is usually relatively short.

MONITORING

Effective and accurate monitoring techniques are required to immobilize hoofstock species successfully. Portable equipment (e.g., capnographs, ABG analyzers, pulse oximeters) facilitate close monitoring of cardiopulmonary performance even under field conditions. The animal should be closely monitored immediately after administration of the immobilization agent(s) and corrective measures initiated if complications such as excitement or prolonged inductions occur. After induction the animal should be placed and maintained in sternal recumbency, the head elevated, and external stimuli minimized by blindfolding and placement of gauze into the external ear canal. Minimally, heart rate, pulse quality (auricular or femoral artery), respiratory rate and pattern, and mucous membrane color should be determined and recorded immediately following induction and every 5 minutes throughout the immobilization period.

Rectal body temperature should be monitored closely throughout the immobilization. Severe hyperthermia (rectal body temperature >41°C) is an emergency situation and is one of the most common complications seen after prolonged inductions and during high environmental temperatures. Clinical signs include tachycardia, tachypnea, and weak peripheral pulses.

A variety of portable monitors are available to check cardiopulmonary performance, and pulse oximetry values, ABG parameters, electrocardiograms (ECGs), and blood pressure (BP) measurements should be recorded regularly throughout the procedure. The ECG leads are placed in a conventional manner and provide information on cardiac rate and rhythm. Arterial BP can be determined directly or indirectly. With the indirect method, a sphygmomanometer is used, and an appropriately sized cuff (width ~40% of limb circumference) can be placed around the base



Fig 56-1 Grevy's zebra (*Equis grevyi*) immobilized with etorphine hydrochloride. The animal is blindfolded to minimize external stimuli, and a pulse oximeter probe has been placed over a lingual artery for continuous determination of relative arterial blood oxygen saturation (SpO_2). A catheter inserted into the nasal passage provides supplemental administration of inspired oxygen.

of the tail or a limb. Direct arterial BP measurements require catheterization of an artery (e.g., facial, auricular, digital). This also allows easy access for collection of arterial samples for ABG analysis, electrolytes, and lactate concentrations. Values for normal ABG parameters in nondomestic hoofstock species are similar to domestic species.

Pulse oximetry allows for continuous, noninvasive monitoring of SpO_2 and is easy to use, especially under field conditions. A transmission probe may be placed on the ear or tongue or a reflectance probe placed at the nasal septum (Figure 56-1). However, the accuracy of pulse oximetry is affected by several factors, including pigmentation at the sampling site, tissue perfusion, and machine calibration based on the human oxygen hemoglobin dissociation curve. Pulse oximetry does not substitute for ABG analysis but has been found useful in detecting trends in arterial oxygen desaturation.^{20,21} Pulse oximetry often overestimates SaO_2 , as indicated by ABG analysis. Pulse oximeters will not assess alveolar ventilation, and end-tidal CO_2 measurements are more accurate in assessing respiratory impairment. End-tidal CO_2 concentrations can be determined with portable capnographs, although accuracy is limited; hoofstock have small tidal volumes when the animal is breathing spontaneously, and therefore alveolar CO_2 concentrations will not be accurately reflected.

CORRECTIVE MEASURES

To avoid potentially life-threatening side effects, the dose of the opioid and synergistic agent(s) should be

carefully determined. A low dosage may result in prolonged recovery times, accompanied by hyperthermia, capture myopathy, and increased risk of trauma, whereas a high dosage may cause severe cardiopulmonary compromise, including respiratory failure.

Proper positioning of the patient is important to avoid myopathies or neuropathies in the recovery period. Immobilized hoofstock should be placed on a flat surface and maintained in sternal recumbency. For prolonged procedures, it is recommended to use a padded surface and support the legs and the neck, especially in large species. The neck should be extended to maintain a patent airway and the head positioned to allow free drainage of saliva and rumen contents in case of regurgitation.

Positioning in sternal recumbency will also help prevent bloat or rumenal tympany, which may result in severe respiratory and cardiovascular compromise. Captive hoofstock should have food withheld for at least 24 hours, if possible, and water 12 hours before immobilization to decrease the prevalence of bloat. Clinical signs include abdominal distention and a rapid, shallow breathing pattern. If the animal is improperly positioned, placement into sternal recumbency and extension of the neck may facilitate resolution of bloat. It may also become necessary to pass a stomach tube or trocharize the rumen.

Hyperthermia is often seen in hoofstock species immobilized during high environmental temperatures or after prolonged physical exertion (e.g., extended chases or induction times).¹⁶ Clinical signs of overheating include tachycardia, tachypnea, and weak, irregular pulses. Rectal body temperatures above 41°C will cause vasodilation, hypotension, cardiovascular collapse, and death if not treated effectively. In cases of hyperthermia, cooling of the animal with cold water, ice packs, cold-water enemas, and intravenous (IV) administration of fluids is recommended, accompanied by close monitoring of plasma biochemical values such as electrolyte concentrations. In severe cases, shock treatment is indicated, including administration of supplemental inspired oxygen. Large species are difficult to manage, and it may become necessary to abort the procedure and reverse the effects of the immobilization agents to restore normal physiologic parameters.

Hypothermia is less often seen than hyperthermia and may be encountered during low environmental temperatures and as a response to immobilizing agents. Small hoofstock species and juvenile animals are particularly sensitive to develop hypothermia because of the previously mentioned conditions. Hypothermia leads to vasoconstriction, decreased circulation, and

in severe cases, shock and death. Treatment of hypothermia includes increasing the body temperature to normal levels. Depending on the size and nature of the animal as well as the immobilizing conditions, heat lamps, blankets, and placement of the animal in a heated stall will be effective.

Respiratory depression is often seen in immobilized hoofstock and is associated with the respiratory depressant effects of the immobilizing agents and the effects of recumbency on cardiopulmonary performance. However, obstruction of the airway caused by improper positioning of the animal after induction, regurgitation of rumen contents, and bloat should also be considered. Maintenance of the animal in sternal recumbency, extension of the neck, and inspection and clearance of the oral cavity of rumen contents will minimize complications associated with upper airway obstruction. Hypoventilation is more pronounced if the animal is placed in dorsal recumbency. If the procedure requires the animal to be in dorsal recumbency, endotracheal intubation and intermittent positive-pressure ventilation (IPPV) are recommended.

To minimize the cardiopulmonary depressant effects of opioid agents and improve the quality of induction and maintenance of anesthesia, carfentanil, etorphine, and thiafentanil are frequently combined with synergistic agents. Ideally, these agents should have minimal cardiopulmonary depressant effects and should also be reversible. For animals determined to be in too deep a plane of anesthesia, the opioid can partially be antagonized, and cardiopulmonary parameters often improve.

Pulse oximetry readings less than 85% to 90% and PaO_2 values less than 60 mm Hg indicate hypoxemia, and the animal requires either assisted ventilation or supplemental oxygen, depending on the severity of respiratory depression. Because all opioid agents and combinations of opioids with synergistic agents are associated with often-pronounced respiratory depression, corrective and supportive measures include nasal or tracheal insufflation with 100% oxygen. Nasal insufflation with oxygen should be initiated immediately after induction and should continue into the recovery period. This can be accomplished by insertion of an appropriately sized nasal catheter into the nasal passage or by tracheal intubation and placement of tubing into the endotracheal tube, followed by administration of oxygen at a flow rate of at least 3 L/min for small ungulates and up to 15 L/min for large species.^{3,20} For routine immobilizations, use of a nasal tube is recommended, whereas for prolonged procedures and in cases of severe respiratory compromise (e.g., decreased respiratory rate, apnea), endotracheal intubation is necessary to control the airway and assist ventilation through IPPV.

To reduce the effects of IPPV on cardiovascular performance, inspiratory pressure should be set at 20 to 30 cm H_2O , the tidal volume at 10 to 20 mL/kg body weight, and respiratory rate at 6 to 10 breaths/min, depending on the size of the patient. In hoofstock, care should be taken not to hyperventilate the animal and induce hypocarbia because this may result in severe bradycardia. In large species, use of a demand valve will deliver oxygen at high flow rates and facilitate administration of an adequate tidal volume while the animal can ventilate spontaneously. In large species especially, respiratory resistance during expiration may be high, and disconnecting the demand valve during exhalation is recommended.

Severe hypoxemia, hypercapnia, or hypotension is often accompanied by tachycardia. If tachycardia is present, the depth of anesthesia, as well as respiratory parameters, should be evaluated for the presence of severe respiratory compromise. To stimulate respiration, administration of doxapram as well as partial reversal of the immobilizing agents may be indicated.

Apnea may result from overdosage of the immobilizing agents and airway obstruction. If apnea occurs, the trachea should be intubated and the animal either manually or mechanically ventilated. Treatment should include administration of IV doxapram (1 mg/kg), and in severe cases, administration of reversal agents.

If the animal develops severe cardiovascular compromise following induction, the plane of anesthesia should be evaluated and decreased if determined too deep. Although hypertension is often seen in anesthetized hoofstock, hypotension may also develop and should be treated with fluid therapy and administration of inotropes to improve circulating fluid volume and cardiac output. Systolic, mean, and diastolic arterial pressures of 120 to 150, 90 to 120, and 80 to 110 mm Hg, respectively, are normal and do not require treatment. Mean arterial blood pressures should always be greater than 60 mm Hg to prevent postanesthetic myopathies.

Administration of calcium borogluconate will increase myocardial contractility, and dobutamine, depending on the dose, will increase cardiac output by increased myocardial contractility and increased heart rate. Dobutamine should be given as a constant-rate infusion, and up to 2 $\mu\text{g}/\text{kg}/\text{min}$ may be reduced to maintenance requirements after resolution of hypotension. Dobutamine is preferable over dopamine in large hoofstock species because it will cause less pronounced increases in heart rate and myocardial work.

Bradycardia is present in small species when heart rates are less than 70 beats/min and in large species less than 50 beats/min. The cause of bradycardia should be determined (e.g., deep plane of anesthesia,

hypoxemia) and corrected. Additional administration of chronotropic agents is recommended. Hypertension is typically seen in anesthetized ungulates after opioid administration and usually is not life threatening. Hypertension has also been associated with increased concentrations of circulating catecholamines with subsequent peripheral vasoconstriction.

Administration of IV fluids is often not practical or indicated for short, routine immobilizations but is recommended for long procedures and in dehydrated patients and those with circulatory compromise. Placement of an IV catheter into the jugular vein is usually easily accomplished following induction of anesthesia. For most hoofstock, a balanced electrolyte solution is recommended, such as lactated Ringer's solution. For maintenance requirements during immobilization, 5 to 10 mL/kg/hr is recommended, whereas fluid deficits or treatment of severe hypotension may require up to 20 mL/kg/hr. In emergency situations or when large volumes of fluids need to be administered, placement of multiple IV catheters may be required.

Capture myopathy has been reported in many hoofstock species and is often associated with prolonged inductions and long chases in free-ranging animals. Detailed information on the pathophysiology of capture myopathy is available.²² To reduce the prevalence of capture myopathy, stress and excessive muscle exertion of the animal before immobilization should be kept to a minimum. This includes selecting effective drugs and dosages to facilitate rapid inductions, minimizing chases under free-ranging conditions, and eliminating excessive stress factors (e.g., noise, unnecessary personnel). Treatment is difficult, and captive animals immobilized within an enclosure are easier to treat than free-ranging animals. Success of treatment depends on early recognition of clinical signs and whether the animal presents in the acute or chronic form of capture myopathy. In the chronic form, treatment is often unsuccessful, and an affected animal often needs to be euthanized. In the acute form, treatment should correct the clinical signs of hypoxemia, hyperthermia, tachycardia, hypotension, and acid-base abnormalities such as metabolic acidosis. Therapy should include administration of oxygen, electrolyte solutions, antiinflammatory agents, glucose, vitamins, and supportive therapy to improve cardiopulmonary performance.

Treatment of a metabolic acidosis requires IV administration of sodium bicarbonate. The dosage of sodium bicarbonate required to treat the metabolic acidosis is determined by the base deficit of the blood;

therefore, acid-base measurements should be performed throughout treatment. To avoid overcorrection of the metabolic acidosis, initial administration of half of the calculated dose is recommended, accompanied by close monitoring of clinical signs and acid-base status.

RECOVERY

During the recovery period, only essential personnel should be present, and external stimuli should be kept to a minimum. The animal should be maintained in sternal recumbency to avoid aspiration pneumonia caused by regurgitation, and the oral cavity should be inspected before reversal. It has been shown that hoofstock species will develop hypoxemia during recovery if insufflation with oxygen is discontinued.²⁰ Therefore, nasal oxygen insufflation should be continued as long as possible into the recovery period.

If the animal has been intubated, the oral cavity should be inspected for regurgitated rumen contents. The endotracheal tube should be removed only when laryngeal reflexes have returned and the animal is swallowing. If regurgitation has occurred, it is best to remove the endotracheal tube with the cuff partially inflated in order to remove food particles from the trachea.

Although the effects of carfentanil, thiafentanil, and etorphine may be effectively reversed by administration of opioid antagonists, in some species the use of carfentanil and etorphine may also result in renarcotization after administration of the opioid reversal agent.^{1,15} The cause is a shorter duration of action of the antagonist compared with the opioid agent. The animal should be monitored regularly for up to 72 hours after reversal of anesthesia, and repeated administration of the opioid antagonist may be necessary if signs of renarcotization are seen, such as head pressing, high stepping, recumbency, pacing, and dulled mentation.

Animals with postanesthetic myopathy or neuropathy will exhibit muscle weakness and inability to stand. Treatment includes IV electrolyte solutions, nonsteroidal antiinflammatory agents, and vitamin E.

To provide smooth, excitement-free recovery, it should also be considered that the analgesic effects of the opioid agent will be reversed after administration of the opioid antagonist. This is particularly important following surgical procedures, and providing effective pain relief into the postanesthetic period with non-opioid analgesic agents is recommended.

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Treatment of Chronic Renal Failure in Nondomestic Felids

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Chronic renal failure (CRF) is a significant cause of morbidity and mortality of older felids in captivity. Chronic renal failure is frequently caused by chronic pyelonephritis, glomerulosclerosis, or amyloidosis. Because the renal changes resulting in CRF are often irreversible, treatment is aimed at slowing the progression of the disease and alleviating signs. Prompt and consistent treatment of CRF can improve the cat's quality of life and longevity.

DIAGNOSIS

Clinical signs of CRF often include polyuria, polydipsia, decreased appetite, weight loss, poor pelage condition, salivation, oral ulceration, vomiting, and dehydration. Nondomestic felids with suspected renal failure should be evaluated with a thorough physical examination and collection of clinical pathology samples. Sample collection should include whole blood for a complete blood cell count (CBC), serum or plasma for chemistry analysis, urine (ideally using cystocentesis) for a complete urinalysis, and a percutaneous kidney biopsy (consider performing a coagulation screen before biopsy) to stage the severity of the disease and monitor response to treatment. Urine should be submitted along with serum for fractional clearance determination as well as bacterial culture.

Clinical pathology may show a mild, nonregenerative anemia with a neutrophilia or normal white blood cell (WBC) count. Serum chemistry results often reveal increases in blood urea nitrogen (BUN), creatinine, phosphorus, and cholesterol and decreases in calcium, potassium, albumin, and total protein. Most nondomestic felids may be trained to allow blood collection with protected manual restraint (or squeeze cage), or with no restraint, and free-catch clean urine collection. This allows serial samples to be collected to monitor response to therapy.

The presence of normal urine specific gravity does not rule out significant glomerular disease (glomerulotubular imbalance). Normal urine protein/creatinine ratio is 0.4 to 0.5. A ratio greater than 1.0 indicates significant proteinuria.

Fractional Excretion Values

In domestic cats the urine protein/creatinine ratio and fractional excretion (FE) of potassium, calcium, phosphorus, and sodium have been shown to increase in CRF. These increases are noted before significant increases in BUN and serum creatinine. In general, the FE ratios have been shown to be more sensitive indicators of renal damage than the urine protein/creatinine ratio. These tests have been used in cheetahs.⁴ The urine protein/creatinine ratio (normal <1.0) requires the collection of only a urine sample, whereas the FE values require a urine sample and matched serum/plasma sample. Fractional excretion ratios are calculated using the following formula:

$$F_{\text{electrolyte}} = \frac{[\text{Serum}_{\text{creatinine}}] [\text{Urine}_{\text{electrolyte}}]}{[\text{Urine}_{\text{creatinine}}] [\text{Serum}_{\text{electrolyte}}]}$$

The following normal ranges have been suggested for cheetah fractional excretion⁴:

FE calcium	0.0-0.13%
FE chloride	0.0-0.20%
FE potassium	0.0-11.70%
FE phosphorus	0.0-16%
FE sodium	0.0-0.07%

TREATMENT

When possible, treatment should be directed against the primary cause of the renal failure, as well as any

complications identified. Careful consideration should be given to discontinuing all nephrotoxic drugs that the felid is currently receiving. A thorough physical examination supplemented with thoracic and abdominal radiography (small, irregularly shaped kidneys) and ultrasonography (increased cortical density and loss of corticomedullary boundary) should be completed to rule out other diseases or causes of chronic inflammation.

Chronic *pyelonephritis* is common in felids and frequently does not cause a significant neutrophilia on the CBC. Cats suspected of having pyelonephritis benefit from long-term antibiotics or pulsed antibiotics. Amoxicillin or amoxicillin plus clavulanate (Clavamox; 20 mg/kg orally [PO] twice daily [bid] or three times daily [tid]) or enrofloxacin (Baytril; 2.5-5.0 mg/kg PO once daily [sid]) has been used successfully in many felids, with treatment for 2 to 4 weeks every 3 months. Reducing the causes of chronic inflammation may be beneficial in slowing the progression of glomerulosclerosis and amyloidosis. Nonsteroidal antiinflammatory drugs (NSAIDs) may be indicated to reduce inflammation byproducts. Colchicine (0.01-0.03 mg/kg/day PO) may reduce serum amyloid A protein release.¹

Common complications of CRF in felids include dehydration, anorexia, proteinuria, hypertension, hypokalemia, hyperphosphatemia, vomiting, and uremia. Aggressive treatment of these complications before or immediately on their appearance may improve the quality of life for the cat.

Fluid Therapy

Dehydration appears to be the most common event that results in decompensation of a felid with CRF. Chronic renal failure results in a polyuria from a decrease in urine-concentrating ability. A compensatory polydipsia offsets the polyuria. If anorexia, vomiting, or diarrhea interrupts the polydipsia, dehydration is likely to occur, resulting in rapid and severe worsening of renal function. Because of the polyuria, daily fluid requirements for cats with CRF are higher than fluid requirements for normal cats.

Fluid therapy is essential to prevent decompensation of the CRF. Felids are more likely to drink water if the water bowl is refilled frequently and the water temperature is cool, but not cold. Ice may be added to the water bowl in hot climates. Some felids will more readily ingest ice cubes with meat juices added. Other cats will drink more water if a small amount of chicken broth (low sodium) is added. Water may be added to

many commercial diets, including chunk muscle meat, and injected into whole-prey items to increase their water content.

Most nondomestic felids may be trained to accept subcutaneous fluid administration with protected manual restraint (or squeeze cage), or with no restraint. Fluid boluses as low as 20 mL/kg, once to four times a week, may significantly improve hydration status in some cats. Cheetahs will usually tolerate the daily administration of 1 to 4 L of subcutaneous fluids. Frequently the benefits of the quick administration of subcutaneous fluids using operant conditioning or manual restraint outweigh the risks of the procedure.

Nutritional Support

Anorexia is a common complication of CRF in felids. Although decreased protein diets (4 g protein/kg/day) are beneficial, it is important to ensure that the cat is consuming sufficient calories and protein. Decreasing the excessive protein in the diet will help decrease the proteinuria. The addition of increased omega-3 polyunsaturated fatty acids will help decrease blood pressure and may have a renal protective effect. Commercial domestic cat renal diets typically contain higher omega-3 ratios, as well as decreased protein, sodium, and phosphorus content. These diets may be gradually introduced to the nondomestic felid and used for up to 75% of the diet (nondomestic felids rarely will eat a diet of 100% commercial renal diets). It is important to use commercial feline renal diets (Hills K/d feline and Purina NF) and not those formulated for dogs.

Anorexia causes catabolism of endogenous proteins, weakness, and lethargy. Every attempt should be made to keep the cat eating. A general guideline is to provide at least 70 kcal/kg/day. Many felids will eat restaurant-quality beef chunk meat, fresh fish, organ meat, or warmed canned domestic cat food. Cheetahs particularly seem to like chicken hearts, warmed Sheba cat food, and chicken baby food. Smaller cats often will eat whole-prey items (rats, mice, quail) when they will not consume normal commercial diets. All prey animals should be treated humanely. Daily food intake should be recorded.

Nondomestic felids may easily be trained to stand on a scale to monitor body mass changes. Diets should be frequently adjusted to prevent loss of body mass. Goals of the nutritional support program should be the maintenance of a stable body weight, stable creatinine level, and stable albumin concentration.

Potassium Supplementation

Hypokalemia is a frequent complication of CRF. Increased urine flow (polyuria) and tubular acidosis frequently cause increased urinary potassium loss. Anorexia (partial or complete) frequently causes decreased dietary intake of potassium. Hypokalemia may be treated with oral supplementation. Care must be taken to avoid hyperkalemia, but this is rare as long as the cat is polyuric. Serum potassium levels should be monitored before and regularly during supplementation. Dosing generally starts at 0.5 to 1 mEq per 4.5 kg body mass bid and is increased by 0.5 mEq/4.5 kg/day until reaching 2 mEq/4.5 kg bid. Renal K (Vet Solutions) and Tumil K (Virbac) are potassium supplements that are available in both a powder and gel formulations.

Potassium supplementation often appears to increase the felid's sense of well-being, with increased activity and interest in its enclosure.

Phosphorus Binding

Hyperphosphatemia is a common complication of advanced CRF caused by decreased renal excretion of phosphorus. In addition to metabolic consequences of electrolyte abnormalities, hyperphosphatemia may cause metastatic calcification of soft tissues when the calcium-phosphorus product exceeds 70 mg/dL. Reducing dietary phosphorus in nondomestic felids is difficult. Most felids cannot be completely converted to low-protein, low-phosphorus renal diets designed for domestic cats (Hills K/d, Purina NF). Often, nondomestic cats may be only partially converted to these diets. However, even a 20% to 50% conversion to a renal diet may make a significant difference.

A number of phosphorus binders are commercially available for use in domestic felids and often may be used in addition to the phosphorus-reduced diets. The phosphorus binder Epakitin (Vetoquinol, EVSCO Pharmaceuticals) is a reasonably palatable powder that may be mixed with food. It may reduce plasma phosphorus and urea concentrations, which may increase the cat's appetite. Amphojel (aluminum hydroxide, 10-30 mg/kg PO tid with meals) is another frequently used phosphorus binder.

Pulse Antibiotics

As noted previously, one of the most common causes of chronic renal disease in felids is chronic pyelonephritis. Without urine cultures, pyelonephritis frequently goes undetected because it may not cause a significant

increase in the WBC count. Many felids will benefit from pulsed doses of broad-spectrum antibiotics. Again, amoxicillin or Clavamox (20 mg/kg PO bid or tid) or Baytril (2.5-5.0 mg/kg PO sid) has been used successfully with a treatment schedule of 2 to 3 weeks four times a year. Serial bacterial cultures of urine collected by cystocentesis are helpful in documenting pyelonephritis and monitoring treatment.

PROTEINURIA

Proteinuria is a frequent complication of CRF in felids and results from damage to the glomerulus or hypertension. Ongoing proteinuria causes additional renal damage as well as a significant loss of protein from the cat's body. Proteinuria may be monitored through the use of microproteinuria test kits, the urine protein/creatinine ratio, and urinalysis. Microalbuminuria generally occurs before increases in the protein/creatinine ratio. In-house test kits designed for use with domestic cats may be used to detect microproteinuria (ERD-Screen Urine Test, Heska; VetTest Urine P:C Ratio, IDEXX). Most urinalysis test strips are not sufficiently sensitive to detect microproteinuria.

Treatment of proteinuria should include reducing the excess protein content of the diet (without causing anorexia). Guidelines for treatment of renal failure in domestic felids suggest that dietary protein should be limited to 4 g/kg/day. In addition to excess protein reduction, proteinuria should be treated with an angiotensin-converting enzyme (ACE) inhibitor. The ACE inhibitors cause vasodilation within the nephron, effectively reducing the hydrostatic pressure, which forces protein through the glomerulus into the tubule. Enalapril (starting dose, 0.5 mg/kg/day PO) is the most frequently used ACE inhibitor. Care must be taken because enalapril is cleared by the kidney, and the dose may need to be decreased in patients with severe renal failure.³ Response to treatment should be monitored by evaluating the level of proteinuria. Newer ACE inhibitors such as benazepril (0.5 mg/kg/day PO) may be better tolerated by felids with CRF.¹

HYPERTENSION

Hypertension is a common complication of CRF, affecting approximately 75% of domestic cats with the disease.² Domestic cats with blood pressure (BP) in excess of 160 mm Hg systolic and 90 mm Hg diastolic are considered hypertensive. Nondomestic felids may be trained using operant conditioning to permit BP monitoring without anesthesia. Indirect BP measure-

ments may be obtained from distal limbs or the base of the tail. Ideally, cats should be trained to remain in lateral recumbency while BP readings are obtained so that the cuff is near the same level as the heart. By using the indirect BP cuff on the tail, the cuff may be positioned at the level of the heart. In addition, BP should be measured during all routine anesthesia procedures. The effect of anesthetics on BP should be considered in the interpretation of results.

If hypertension is documented or suspected, treatment should be initiated. Reducing dietary sodium intake may help decrease BP. Most commercial renal diets are sodium restricted. Enalapril is the most common first-line drug used. Dose must be decreased if the cat is in severe renal failure. A beta blocker such as propranolol may be added if the ACE inhibitor is not sufficient to decrease BP. Untreated hypertension may speed the progression of CRF through damage to the glomerulus and cause blindness.

UREMIA

High BUN may result in clinical signs of uremia, including oral ulcerations, anorexia, vomiting, gastric

erosions, and ulceration. Uremia may be treated using Epakitin (see Phosphorus Binding). Metoclopramide (Reglan; 0.2-0.5 mg/kg PO bid/tid) is an effective antiemetic and increases gastric motility. A histamine-2 receptor blocker such as ranitidine (Zantac; 3.5 mg/kg PO bid) or a proton pump inhibitor such as omeprazole (Prilosec; 0.5-1.0 mg/kg PO sid) combined with sucralfate (Carafate; 0.25-1.0 g PO bid 30 minutes before antacids) may be effective treatments for gastric erosions and small ulcers.³

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